VOLUME 47 July, 1957 NUMBER 7

QK 1 P6 PR

Phytopathology

An International Journal

UNIVERSITY OF MICHIGAN JUL 30 1957 SCIENCE LIBRARY

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONTENTS

Benjamin Minge Duggar, 1872-1956 — J. C. Walker 379

Symposium on Foreign Plant Quarantines and Regulatory Administration:

Redefinition of the principles of plant quarantine and their relation to the current problems — F. A. Sorauf 381

Some broad aspects of federal crop regulatory work — M. R. Clarkson 382

What the amendment to the Insect Pest Act of 1905 implies in relation to the layman and to the scientist — H. S. Dean 384

The movement of plant pathogens — W. H. Wheeler 386

The discovery of important diseases before they move from one country to another — A. J. Riker 388

The effect of virus infection on yield and chemical composition of Ladino clover — K. W. Kreitlow, O. J. Hunt, and H. L. Wilkins 390

Temperature and seasonal effects on seedling reactions of Barley varieties to three races of Puccinia graminis f. sp. tritici — F. L. Patterson, R. G. Shands, and J. G. Dickson 395

Venturia inaequalis (Cke.) Wint. XII. Genes controlling pathogenicity of wild-type lines — D. M. Boone and G. W. Keitt 403

Lamont, a new variety of rescuegrass resistant to head smut — Howard W. Johnson 409

Studies on bacterial blight of lima bean — Maung Mya Thaung and J. C. Walker 413

Prune crinkle leaf — Earle C. Blodgett 418

Factors influencing the elimination of loose smut from barley by water-soak treatments — L. E. Tyner 420

Further study of the preservation of Puccinia uredospores — Eugene L. Sharp and Frederick G. Smith 423

Inhibition of Fusarium oxysporum f. lycopersici by a species of Micromonospora isolated from tomato — Glenn E. Smith 429

Control of citrus fruit russet in Florida with Zineb — Fran. E. Fisher 433

Phytopathological Notes:

Pathogenicity of Idriella lunata on strawberry — Paul E. Nelson 438

The morphology of the Sclerotium of Claviceps paspali — Robert B. Stewart 444

An improved method of inoculating barley leaves with Rhynchosporium secalis — W. P. Skoropad 445

Fruit decay and twig cankers on peach — M. A. Smith and G. B. Ramsey 445

Association between Rhizoctonia and yellow coloration of Easter lily bulbs — Frank P. McWhorter 447

Streptomycin foliage sprays and control of bacterial spot of peach — Robert H. Daines and Reed Gray 448

Symptoms of chrysanthemum flower distortion, dodder transmission of the virus, and heat cure of infected plants — Philip Brierley and Floyd F. Smith 448

Sorghum grain, a medium suitable for the increase of inoculum for studies of soil-borne and certain other fungi — Marvin D. Whitehead 450

Report and abstracts of the 1957 meeting of the Potomac Division of the American Phytopathological Society 451

Book listings 454

News

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

Here's the Story of Expansion In ORTHO Applied Research . . .

ORTHO plant pathology research tests newly developed fungicides in an expanding program of applied biological laboratory and controlled-plot research

Long recognized as a leader in fundamental pesticide research, ORTHO is continually increasing its emphasis on applied biological research.

In addition to fundamental research and control laboratories, ORTHO now has a total of 13,200 square feet of modern biology laboratories staffed by biologists and plant pathologists, 10,800 square feet of experimental planting area under glass, and 28 acres of experimental plots—principally at Richmond and Moorestown.

Extensive greenhouse and field testing of new fungicides, bactericides and nematocides—developed by ORTHO fundamental research, which is also carrying on its year-around work on insecticides, viricides, and herbicides—is now in progress on many economic crops and ornamentals.

(Incidentally, the use of ORTHOCIDE by independent researchers has increased consistently since the highly favorable report issued by the APS Temporary Advisory Committee on Collecting and Disseminating "Results on 1955 Fungicide Tests.")

You are cordially invited to use proven ORTHO pesticides—such as ORTHOCIDE—in your work, as well as new fungicides tested by our own biological research laboratories, as they become available.

TMs Reg. U.S. Pat. Off.: ORTHO, ORTHOCIDE

For complete technical information on ORTHOCIDE and ORTHO's newest fungicides and other pesticides, write Dr. Thompson if in the 11 Western States, Dr. Wessel if in the Midwest or East . . .

Dr. R. B. Wessel
Field Research Supervisor, East
California Spray-Chemical Corp.
Box 118
Moorestown, New Jersey

Dr. R. K. Thompson
Field Research Supervisor, West
California Spray-Chemical Corp.
Lucas and Ortho Way
Richmond, California

ORTHO

BENJAMIN MINGE DUGGAR, 1872-1956

J. C. Walker

At a meeting of plant pathologists during the annual conclave of the American Association for the Advancement of Science in Baltimore, Maryland, in December, 1908, the American Phytopathological Society was organized with the election of the following officers: L. R. Jones, *President;* A. D. Selby, *Vice-President;* C. L. Shear, *Secretary-Treasurer;* J. B. S. Norton and B. M. Duggar, *Councillors.* The death of Benjamin Minge Duggar at the age of 84 at New Haven, Connecticut, on September 10, 1956, closed a remarkable career of service to botany, plant physiology, plant pathology, and medicine. The space allotted to this biographical sketch is entirely inadequate to do justice to Duggar's contributions to various branches of science; therefore the bearing of his work on the field of plant pathology will be given major emphasis.

He was born at Gallion, Alabama, on September 1, 1872, the fourth of 6 sons of Reuben H. and Margaret Louisa (Minge) Duggar. His father was a practicing physician who had served as a surgeon in the Confederate Army during the Civil War. Trained in a private preparatory school under tutors, Duggar attended the University of Alabama from 1887 to 1889 and the Mississippi A. and M. College from 1889 to 1891, receiving the B.S. degree with first honors from the latter institution in 1891. He then attended Alabama Polytechnic Institute, working under the direction of G. F. Atkinson and receiving the M.S. degree in 1892. As an undergraduate at Mississippi A. and M. College, he assisted in experimental work in connection with the introduction of forage grasses new to the area. In 1892 and 1893 he was in charge of the Canebrake Station at Uniontown, Alabama, where this interest continued. In 1894 and 1895 he engaged in graduate work under the supervision of W. G. Farlow at Harvard University and served also as Assistant in botany at Radcliffe College. He completed the requirements at Harvard for the B.A. and M.A. degrees, receiving the latter degree in 1895. As Assistant Botanist at the Illinois State Laboratory of Natural History at Urbana (1895–1896), he was engaged in a study of certain bacterial and fungus parasites of insects. During the next 5 years he was on the staff at Cornell University, serving successively as Assistant Cryptogamic Botanist, Instructor, and Assistant Professor of Plant Physiology. During this period he completed his graduate training under the direction of G. F. Atkinson and received the Ph.D. degree in 1898. He published papers on diseases of pear, stone fruits, and sugar beet and began an extensive study of the genus *Rhizoctonia.* Several months in 1899–1900 were spent in Europe at several laboratories including that of Julius Kühn, who was the first to describe *Rhizoctonia solani.* A monograph comprising Duggar's studies of *Rhizoctonia* appeared in 1915.

In 1901–1902 he was Physiologist in the Bureau of Plant Industry, United States Department of Agriculture, where he began studies with cultivated mushrooms and with the cotton root rot fungus. He continued these studies after becoming Professor of Botany at the University of Missouri in 1902. He discovered the conidial stage of the cotton root rot fungus and transferred the species from *Ozonium* to *Phymatotrichum.* He pioneered in the artificial cultivation of mushroom spawn in this country. His first monograph on this subject was published as a Bureau of Plant Industry Bulletin in 1905. At the St. Louis Exposition in 1903, a grand prize was granted to the University of Missouri for his exhibit on the culture of mushrooms and other fungi. He was granted leave to study in Europe and North Africa in 1905–1906. During this period he gave special attention to the culture of truffles and mushrooms. His well-known book, *Mushroom Growing,* was published in 1915. Before he left the University of Missouri, he had practically completed the manuscript for the first American text book in plant pathology, *Fungous Diseases of Plants,* which appeared in 1909.

In 1907 Duggar was called to the chair in plant physiology at Cornell University. He remained there

BENJAMIN MINGE DUGGAR
1872–1956

PHYTOPATHOLOGY for June (47: 321-378) was issued June 28, 1957

for 5 years until 1912, when he became Professor of Plant Physiology in charge of the Graduate School in the Henry Shaw School of Botany at Washington University and Missouri Botanical Garden in St. Louis. He attracted numerous graduate students, many of whom became well-known figures in the field of plant pathology. During this period he initiated an extensive series of researches on physiology of the fungi. It was here also that he began his studies on tobacco mosaic and other virus diseases. His fundamental studies on the size and properties of tobacco mosaic virus particles are important landmarks in the steady progress of events during the twenties and thirties which preceded the isolation of discrete protein crystals by W. M. Stanley in 1935. The breadth of his training and ability is shown by the fact that during World War I (1917–19) he served as Acting Professor of Biological Chemistry in the Medical School of Washington University in order to relieve P. A. Shaffer, who had been called into special war duties.

In 1927, Duggar was called to the University of Wisconsin as Professor of Physiological and Economic Botany. In 1929 his duties were broadened to include a part-time professorship in the Department of Plant Pathology. Although he gave no formal courses in plant pathology, he served as major or minor professor to many graduate students in this discipline. Most students majoring in the subject took his course in the physiology of the fungi. His broad knowledge of botany, his encyclopedic memory, his original and brilliant mind combined with a gentlemanly, sympathetic, and helpful attitude made for him an unrivaled position as an adviser to graduate students in various branches of plant science.

Duggar's research program was continuously alive and productive. In addition to his program on plant viruses, he collaborated with A. J. Riker in studies on the fundamental aspects of atypical growth with special reference to crown gall. He also shared leadership with Farrington Daniels, Professor of Physical Chemistry, in a study of the energy relations in photosynthesis.

In his seventy-first year (1943) he became Emeritus Professor at the University of Wisconsin. In 1944 he accepted an appointment with the Lederle Laboratories Division of the American Cyanamide Company at Pearl River, New York, as consultant in mycological research and production. Although this new responsibility began as an emergency effort in the midst of World War II, it ripened into a productive career that extended for slightly more than twelve years until the time of his death. It was early in this period that he began his extensive search among the soil fungi for species that produced effective antibiotics. Out of this came the discovery of the new species *Streptomyces aureofaciens* Duggar; this work became the basis for the manufacture and widespread use of aureomycin and its derivatives, not only as a drug but also as a widely used disinfectant in the processing of poultry and as a supplement in animal feeds.

During his long career, Duggar occupied many important extracurricular positions. His part in the organization of the American Phytopathological Society has been mentioned. In 1907 at the Chicago meeting of AAAS, he participated in the organization of the American Society of Agronomy and became one of its two first vice presidents. He belonged to numerous other scientific societies, including the National Academy of Sciences and the American Philosophical Society. He served as president of the Botanical Society of America in 1923 and of the American Society of Plant Physiologists in 1947. He was vice president of Section G, AAAS, in 1925, and of the American Society of Naturalists in 1928. He was Chairman of the Division of Biology and Agriculture, National Research Council (1925–26). He was a Trustee of the Marine Biological Laboratory at Woods Hole, of the Oceanographic Institute at Woods Hole, and of the Bermuda Biological Station. He was editor of plant physiology for BOTANICAL ABSTRACTS during its entire period of publication (1917–1926), and he continued in the same capacity for Biological Abstracts from the time of its inception (1926) until 1933. He was editor of the Proceedings of the International Congress of Plant Science (2 volumes) when that Congress met at Cornell University in 1926 and of a 2-volume work on *Biological Effects of Radiation* issued in 1936.

Three institutions at which he had served conferred honorary degrees upon him (University of Missouri, L.L.D., 1944; Washington University, Sc.D., 1953; University of Wisconsin, D.Sc., 1956). As the discoverer of aureomycin, his fame extended far beyond the field of plant science. He accepted speaking engagements before medical science groups in many parts of the world. He was elected a Fellow of the International College of Surgeons in 1952. He was the recipient of the Medal of Honor of Public Education of Venezuela in 1951. He was received in private audience and given a special medal by Pope Pius XII. In Japan has was invited to a private audience by the Emperor.

Dr. Duggar was united in marriage in 1901 with Marie L. Robertson of Ithaca, New York, who died in 1922. In 1927 he married Elsie Rist of St. Louis, Missouri, who survives him. He is also survived by two sons and four daughters.

Professor Duggar was a lover of nature and of the out-of-doors. He continued to engage in fresh-water and deep-sea fishing until a few weeks before his final illness. His favorite indoor sport was bowling, in which he participated until less than 2 years before his final illness.

Few scientists have had the privilege of serving so well and so productively over a period of nearly seven decades. We in plant pathology have gained immeasurably from having had such a great and gentlemanly figure in our midst. Many will remember the special tributes extended to him at the annual banquet of the American Phytopathological Society at Memphis in December, 1950.

REDEFINITION OF THE PRINCIPLES OF PLANT QUARANTINE AND THEIR RELATION TO THE CURRENT PROBLEMS [1]

F. A. Soraci [2]

A quarantine is a restriction, imposed by duly constituted authority, whereby the production, movement, or existence of plants, plant products, animals, animal products, or any other article or material, or the normal activity of persons, is brought under regulation in order that the introduction or spread of a pest may be prevented or limited, or in order that a pest already introduced may be controlled or eradicated, thereby reducing or avoiding losses that would otherwise occur through damage done by the pest or through a continuing cost of control measures.

Since a quarantine is a restriction, it must have a proper justification. The Principles of Plant Quarantine provide such justification and they provide also a fair and reasonable procedure for administration of the necessary regulations.

The National Plant Board was organized in 1925 "to improve and unify quarantine and regulatory activities. . . ." In 1927 a committee was appointed to draft the principles of plant quarantine and to promote uniformity in promulgation and administration of quarantines between state and federal agencies. The statement presented by that committee, composed of S. P. Fracker and W. A. McCubbin, was formally approved by the 4 Regional Plant Boards and accepted with little revision as *National Plant Board Principles of Plant Quarantine* in November 1930.[3] The *Principles* are still accepted after a lapse of more than 25 years.

As recently as 1952, the National Plant Board appointed a committee to study the *Principles* and to recommend desirable revisions. That committee, after careful study, proposed several changes in the interest of clarity, brevity, and better grammatical usage, but they were not accepted, since the majority of the state quarantine officials were of the opinion that the *Principles* in their present form had been well established and were well understood.

In view of this background, it would be presumptuous on my part to attempt a redefinition; rather let us try to restate them briefly.

A *definition* of a plant quarantine has been given.

There should be a *basis in logic;* resort to plant quarantine regulations by public authority is logical because the ends to be obtained are in the interest of public welfare, and the measures could not be undertaken by private individuals or groups.

There must be *necessity*, i.e. the pest must offer a threat to substantial interests, a measure involving less interference with normal activities must be unavailable, the objective must be reasonable of expectation, and the economic gains expected must outweigh the cost of administration and of interference with normal activities.

Of course, there must be *legal sanction*, i.e. adequate law and authority.

There must be *validity;* the quarantine should be used only as an aid in exclusion or control of the pest and not for some unstated objective such as hindrance of trade.

Public notice is desirable when possible.

In *scope*, the restrictions must be only those that are believed essential to the attainment of the stated objectives.

If *eradication* is the reasonable objective, then restrictions might properly be severe.

If *retarding spread* is the objective, the restrictions must be only those that are consistent with the continuance of the pest in the infested area and hindrance of its possible spread to other areas.

The need for *co-operation* with other local, state or federal authorities should be recognized.

The *co-operation of the public* must be sought, rather than depending exclusively on police powers, the imposition of penalties, or resort to court action.

The quarantine statement should be phrased *clearly* and *simply*.

Information service should be provided so that the public is advised as to objectives of the quarantine and the reasons for restrictions.

Research must be encouraged so that the quarantine might be made more efficient and restrictions lessened where possible; however, the need for research should not be permitted to delay establishment of a quarantine, thereby jeopardizing the objective.

There is recognized the obligation to *modify* the quarantine as conditions change or as further facts become available.

Repeal or substitution of other measures should be considered promptly if it becomes evident that the desired end is not possible of attainment by the restrictions adopted.

Finally, there is recognized the need for notice to *parties at interest*, particularly organizations representing the public involved in the restricting measures and federal and state authorities.

These are the principles of plant quarantine recognized by the 48 States. To relate these principles to current problems would take much more time than can be given to the subject at this point. I can only make some broad statements and give you some personal opinions. In general, the principles have served their purpose over the years. No one concerned with plant

[1] Invitational paper presented December 6, 1956 at the Symposium on Foreign Plant Quarantines and Regulatory Administration at the 48th Annual Meeting of the American Phytopathological Society at Cincinnati, Ohio.

[2] Director, Division of Plant Industry, New Jersey State Department of Agriculture.

[3] National Plant Board Principles of Plant Quarantine. 1931. 3p. (Mimeographed).

quarantine affairs would consider the establishment of a quarantine without consulting these principles. The form of a state quarantine must, of necessity, conform with the requirements of law within that state; therefore, uniformity of wording must not be expected. The principles have served well and are adequate for the removal or modification of bad quarantine regulations.

To Fracker, McCubbin, Headlee, O'Kane, and many others who helped to bring into proper focus the basic needs of plant quarantines, we must give full credit for the establishment of sound principles that have withstood the ravages of our times. From time to time we are confronted with new pest problems, changes in business methods and concepts, changes in the economy, and changes in methods of transportation, but the soundness of the principles has not yet been really challenged.

SOME BROAD ASPECTS OF FEDERAL CROPS REGULATORY WORK [1]

M. R. Clarkson [2]

It is estimated that plant diseases alone cost the United States 3 billion dollars a year. These are staggering figures. Yet no one can say how much greater the losses might be were it not for the joint efforts of plant pathologists and regulatory workers who are constantly striving to improve our ability to cope with this threat to crops. Many successes over plant diseases of various kinds have depended on the close cooperation and interrelationship of the several scientific disciplines. Perhaps there is no better evidence of this interdependence than in the research and regulatory activities designed to protect the nation's agriculture. Nowhere in agriculture are research results applied more rapidly than in the prevention, control, and eradication programs.

Experience has taught us that, in the long run, it is cheaper to live *without* damaging diseases or other pests than to live *with* them. That is why the regulatory worker, whether in animal disease or plant pest control, is making every effort to prevent entry of dangerous foreign pests and is constantly striving towards the goal of eradication of those already here.

Basically, it was this philosophy that led to the passage of the Plant Quarantine Act of 1912. With its later amendments, this Act provided for many safeguards against entry and establishment of foreign pests, as well as against the domestic spread of those introduced.

The regulatory program as we know it today has evolved from that basic act. Under existing state and federal laws, cooperative pest control and eradication programs within a state are carried on under state authority. Although an outbreak of a particular pest may be local in scope, its suppression may provide protection to other areas of the nation not yet infested.

The states have a vital role in such regulatory programs. With regard to plant-pest control work, they have responsibility for preventing the spread of seriously injurious pests by the use of quarantines or other suitable means in the absence of federal action; for developing and administering quarantines within the state, including those that supplement interstate action; and for providing independently, or with other states, for the control of pests that have reached the normal limit of their range. This discussion, however, is concerned with the general aspects of the federal crops regulatory programs.

Federal responsibilities break down into 6 major categories. They are 1) preventing introduction of damaging pests into the United States; 2) preventing the spread between states; 3) developing, correlating, and evaluating pest information; 4) working cooperatively with foreign countries, particularly close neighbors, in the control of crop pests to prevent their crossing our boundaries; 5) conducting pest eradication or control programs on federal property; and 6) cooperating with the states in programs involving joint responsibility.

Without quarantine barriers, the United States could readily become the habitat for a host of plant diseases that are not now known in this country. There are still many important plant pests in other parts of the world that must be kept out of the United States. There is the citrus canker—a highly destructive disease of citrus and one that has been eradicated from the United States after long and expensive effort. There are many virus diseases, such as yellow wilt of sugar beets and turnip yellow mosaic, which have not yet made their way to the United States.

Under the provisions of the quarantine act, plants and plant products from overseas may be prohibited entry or be allowed to be brought in under regulated entry, subject to inspection and treatment as conditions of entry.

The Department maintains an agricultural "Ellis Island" at several major ports—including Hoboken, New Jersey, for northeastern ports; San Francisco, California, and Seattle, Washington, for arrivals on the Pacific Coast; and Miami, Florida, and Laredo, Texas, for arrivals in the South. These facilities receive imported plants for intensive inspection and for whatever treatment is required.

[1] Invitational paper presented December 6, 1956, at the Symposium on Foreign Plant Quarantines and Regulatory Administration at the 48th Annual Meeting of the American Phytopathological Society in Cincinnati, Ohio.

[2] Deputy Administrator, Agricultural Research Service, U. S. Department of Agriculture.

The Plant Quarantine Act is credited with having cut in half the number of introductions of damaging foreign insects. It is safe to say we have gained as much with regard to plant diseases. This has been done in spite of vastly increasing commerce, with more and more people and goods moving about the world. Modern transportation brings this country close in time to distant lands—and to damaging plant pests that imported goods may carry. The speed of modern travel increases the opportunities for the pests to arrive here in a living state.

The first line of defense against dangerous foreign pests is constant alertness on the part of quarantine inspectors at the ports. During the 12-month period ending May 31, 1956, some 84,000 planes and 46,000 ocean vessels arrived from abroad, and 17,262,000 vehicles crossed the Mexican border. Plant quarantine inspectors also checked 10,000 planes in Hawaii and 2,300 in Puerto Rico before departure of the planes for the mainland. When inspected, 37,000 of these planes were found to carry prohibited or restricted plant materials. Inspection activities at seaports yielded 244,500 lots of contraband plant materials and 17,500 plant pest interceptions.

PREVENTING THE SPREAD OF PLANT PESTS BETWEEN STATES.—Man enlarges the problem of natural spread of disease through shipment of seeds, cuttings, and nursery stock. For example, the spreading decline of citrus has reached out beyond its normal limits through distribution of infected nursery stock before preventive measures were developed. The European chafer and the Japanese beetle can be spread through the careless movement of soil attached to nursery stock and by other means. Fruit flies are carried afar through movement of infested fruit.

By enforcing rigid controls, it is sometimes possible to confine a pest to one state or even one area. For example, the golden nematode exists in this country on approximately 4,000 acres of agricultural land on Long Island. Strict controls, exercised with the full cooperation of potato growers, the State of New York, and the U.S. Department of Agriculture, have kept the nematode within the limits of the island, and it is on the decline there. It is possible now to look ahead to eventual eradication.

DEVELOPING, CORRELATING, AND EVALUATING PLANT-PEST INFORMATION.—Within the scope of regulatory functions, the Agricultural Research Service receives information from foreign countries on pests that are indigenous to them and on outbreaks that reach dangerous proportions. By having a central agency receive, coordinate, and evaluate this kind of information, it is possible for our port plant quarantine inspectors to know what to expect from materials coming from different parts of the world. They can know what to look for and how to look for it.

The Agricultural Research Service also develops, correlates, and evaluates information on pests here at home. This is done in cooperation with the states and other agencies. The Economic Insect Survey and the Plant Disease Warning Service are the results of this organized reporting. They summarize the reports received and show the intensity and extent of insect infestations and plant disease incidence. Both reports go to agricultural workers, including industry groups, so that they can formulate control plans and make arrangements for distribution of needed materials in areas where serious outbreaks may be expected. Recently a Plant Disease Warning Service letter went out telling about the appearance of the soybean cyst nematode in western Tennessee, some 400 miles from North Carolina, the only other state in which it has been reported.

WORKING COOPERATIVELY WITH FOREIGN COUNTRIES IN THE STUDY AND CONTROL OF THREATENING PESTS.—In Mexico, current projects include research on rust-resistant varieties of small grains; both research and control programs for the pink bollworm, the Mexican fruit fly, and the citrus black fly; and a cooperative control effort on the khapra beetle. Cooperative projects with Canada include research on rust-resistant varieties of grains and methods of preventing spread of the white pine blister rust and the gypsy moth. Department people have completed an initial survey in Central America and the West Indies to locate the Mediterranean fruit fly and to prevent its reintroduction to this country from any southern countries. The survey is being continued by certain of the Central American countries with technical advice being furnished by the Agricultural Research Service.

Recently, the Plant Quarantine Division embarked on a new undertaking for the further protection of this country's agriculture. In 1951, on invitation from the government of The Netherlands, the Plant Quarantine Division sent inspectors to Holland at the time of packing bulbs for shipment to the United States. Because of the success of this "country-of-origin" inspection, the work has been carried on each succeeding year. The Belgian Government requested similar work in 1952, and France requested it in 1954. The arrangement makes it possible to prevent entry of diseased or infested bulbs and saves the foreign government, as well as the American buyer and the exporter, time and money.

CONDUCTING PEST ERADICATION OR CONTROL ON FEDERAL PROPERTY.—This concerns primarily the control of insects and diseases attacking federally owned grazing lands or forests. For example, the blister rust, a threat to the white pine forests both of the eastern United States and of the great mountain regions of the Northwest, is a problem for federal action. This is a part of the work of the Forest Service.

COOPERATING WITH THE STATES IN PROGRAMS INVOLVING JOINT RESPONSIBILITY.—These are illustrated by the federal-state programs on barberry eradication, on the control of peach mosaic, and on the eradication of phony peach virus, Hall scale, the burrowing nematode, and other pests.

Congress has recognized in recent years the need for and the advantages to be gained through prompt action in dealing with new introductions. It has made

a contingency fund available to ARS to use in cooperating with states and the industry affected when emergency or unexpected situations arise.

This discussion has been a broad view of the federal regulatory programs on crops. For the purposes of this symposium, it would be well to consider some of the needs yet to be satisfied in regulatory work.

Although this Government finally has arrived at the point where it has a reasonably adequate port-of-entry inspection, there is a need for the program to be kept flexible and strengthened as changing conditions warrant.

Surveys, disease and insect, need to be strengthened and made more comprehensive so that any incipient infestation by an insect or infection by a pathogen may be detected while the area involved is small and controllable. Frequently, this would provide an opportunity for eradication at reasonable cost and without serious impact on the industry.

To lend strength to regulatory and control efforts, it is becoming more and more urgent that this country develop a thorough knowledge of agricultural pest problems that exist elsewhere in the world. Pest-control officials were able to move effectively against the Mediterranean fruit fly in Florida partly as the result of research work accomplished in Hawaii. Since there was no opportunity to study this insect here, ARS enlarged the laboratories in Hawaii where 3 damaging flies—the oriental fruit fly, the melon fly, and the Mediterranean fruit fly—could be studied. The information developed in Hawaii was quickly adapted to the Florida situation, permitting specialists to move ahead with assurance that they were doing the right thing.

Research is needed on such things as the treatment of plants against nematode infections and the treatment of plants and seeds having fungus and bacterial diseases. More research on virus diseases of plants is necessary. And far more information on plant pathogen and nematode distribution in this and foreign countries is needed for more effective plant quarantine work.

In every scientific field, however, the greatest need today probably is for more professional people. Certainly this is true in the federal regulatory programs. The plant quarantine inspector must be informed in many fields, because on him rests the responsibility of protecting agriculture from devastating insects and diseases. We urgently need to encourage more of the talented students to become plant pathologists, entomologists, nematologists, and botanists to keep pace with the fast-growing demands of modern agriculture. These young people need your direction, encouragement, and inspiration.

WHAT THE AMENDMENT TO THE INSECT PEST ACT OF 1905 IMPLIES IN RELATION TO THE LAYMAN AND TO THE SCIENTIST [1]

H. S. Dean [2]

The plant pathologists are concerned with the legislative proposal to amend and revise the so-called Insect Pest Act of March 3, 1905 (33 Stat. 1269; 7 U.S.C. 141-144), and with what the revisions will mean to the layman and to the scientist. Such a proposal was developed in the Department, and after it was cleared through various other departments it went to Congress. Since the proposal died with the 84th Congress, it is planned to resubmit it during the first session of the 85th Congress.[3]

ACTS NOW IN EFFECT.—In carrying out the responsibilities of the Plant Quarantine Division to protect the agriculture of this country from introduction, establishment, and spread of pests and diseases injurious to plants and plant products, several statutory tools are available but others are needed and some need modernization. The federal quarantines at present rely primarily on authority contained in the Insect Pest Act already referred to; the Plant Quarantine Act of August 20, 1912 (37 Stat. 315, as amended; 7 U.S.C. 151-154, 156-161, 162-164a, 167); the Mollusk Act of September 22, 1951 (65 Stat. 335; 7 U.S.C. 441); and the Mexican Border Act of January 31, 1942 (56 Stat. 40; 7 U.S.C. 149). In addition, Section 102 (a) of the Department of Agriculture Organic Act of 1944, as amended (58 Stat. 735, as amended; 7 U.S.C. 147a (a)), authorizes the Secretary of Agriculture independently or in cooperation with states or political subdivisions thereof, with farmers' associations and similar organizations, with individuals, and with the Government of Mexico or local Mexican authorities to carry out operations or measures to eradicate, suppress, control, or to prevent or retard the spread of listed plant pests.

The Insect Pest Act prohibits the knowing movement, except for scientific purposes under regulations of the Secretary of Agriculture, from any foreign country into the United States, or interstate, of any insect in a live state which is notoriously injurious to cultivated crops.

[1] Invitational paper presented December 6, 1956, at the Symposium on Foreign Plant Quarantines and Regulatory Administration at the 48th Annual Meeting of the American Phytopathological Society at Cincinnati, Ohio.

[2] Plant Quarantine Division, Agricultural Research Service, U. S. Department of Agriculture.

[3] This Act was approved May 23, 1957, as Public Law 85-36.

The Plant Quarantine Act imposes certain requirements with respect to the importation of nursery stock, authorizes the Secretary of Agriculture to extend such requirements to the importation of other plants and plant products, and authorizes the imposition of quarantines and restrictions upon the movement of plants, plant products, stone or quarry products, and other articles of any character whatsoever from any quarantined state, territory, or district into or through any other state, territory, or district, to prevent the spread of insect infestations or plant diseases. This Act authorizes the inspectors of this Department to stop and search, without a warrant, persons coming into the United States and any vehicles, receptacles, boats, ships, or vessels coming from any country or moving interstate, upon probable cause to believe they carry or contain articles the entry or movement of which in foreign or interstate commerce is prohibited or restricted by the Act of any order thereunder. The Act further authorizes the inspectors to seize and dispose of any articles found to be moving or to have been moved in interstate commerce or to have been brought into the United States in violation of the Act or such order.

The Mollusk Act is intended to prohibit the importation of terrestrial or fresh water mollusks injurious to agriculture. It authorizes regulations governing the inspection and treatment of produce, baggage, salvaged war materials, and other goods entering the United States to prevent the entry of such mollusks.

The Mexican Border Act provides for the regulation of the entry into the United States from Mexico of railway cars and other vehicles, freight, express, baggage, and other materials which may carry insect pests and plant diseases and, when necessary, the disinfestation of such vehicles and materials.

From the legislation thus briefly summarized, it is readily apparent that there are statutory deficiencies. The American Phytopathological Society has long been cognizant of one such deficiency of particular interest to the membership of the Society, namely, the indiscriminate importation of living cultures of plant pathogens (see PHYTOPATHOLOGY 26: 498. 1936). In addition to the legislative deficiency with respect to plant pathogens such as protozoa, fungi, viruses, and nematodes, the legislation cited does not bar the movement into or through the United States, or interstate, of insects and mites *not notoriously* injurious to cultivated crops. And under present legislation, there is no legal authority to take emergency action, except in so far as the Mollusk or Mexican Border Acts would apply.

PROPOSED FEDERAL PLANT PEST ACT.[3]—Much thought has been given during the past several years to the development of a Federal Plant Pest Act to replace the Insect Pest Act of 1905 and to complement the authority contained in the Plant Quarantine Act. Such legislation would help to prevent the unrestricted entry of plant pathogens and provide for their importation for scientific purposes under permit.

The proposed legislation is analyzed as follows:

Section 1 is the title of the act, i.e. "Federal Plant Pest Act."

Section 2 contains definitions. "Plant pest" means any living stage of any insects, mites, nematodes, slugs, snails, other invertebrate animals, protozoa, bacteria, fungi, other parasitic plants or reproductive parts thereof, viruses, any organisms similar to or allied with any of the foregoing, or any infectious substances which can directly or indirectly injure or cause disease or damage in any plants or parts thereof, or any processed, manufactured, or other products of plants.

Section 3 provides authority to promulgate regulations to govern importation or interstate movement of plant pests. Whereas the proposed bill would require permits for the movement into or through the United States, or interstate, of any plant pest, it would be impractical to attempt to establish regulations specifying the conditions under which each of the pests might be imported or moved interstate. It has been the practice in the enforcement of the Insect Pest Act and the Mollusk Act and in the voluntary efforts to regulate the movement of plant pathogens to consider each case on its merits. Such procedure has been found satisfactory and no great impediment to the public or to research workers. This procedure allows qualified research workers to obtain and use material necessary for their investigations under conditions which will prevent the dissemination of the pests and to allow the movement of plant pests for other purposes only under such circumstances as do not involve a hazard from such dissemination. It is the intent of the Department that a similar sound policy will prevail if this proposal is enacted into law.

Section 4 makes plant pests nonmailable except by permit. The precedent for this lies in the Insect Pest Act. Simple procedures have been developed to make this requirement readily workable as explained in the next paper.[4]

Section 5 authorizes the Department to take emergency action where the movement of plant pests has not been anticipated and made subject to a plant quarantine or order issued under the Plant Quarantine Act. Protection is provided for the owner of any product, article, or means of conveyance against destruction, export, or return to point of origin if less drastic action will prevent the dissemination of plant pests. It provides for just compensation to the owner in the case of destruction or other disposal of any product, article, or means of conveyance where such destruction or disposal is not authorized by the Plant Quarantine Act or the proposed act. The compensation provision is included in the proposed legislation to avoid constitutional questions with respect to denial of due process of law.

[4] Wheeler, W. H. 1957. The movement of plant pathogens. Phytopathology 47:386-388.

Section 6 provides authority to promulgate regulations requiring inspection broader than that contained in the present legislation including necessary inspections of plants and plant products and articles of any character whatsoever, and means of conveyance moving into or through the United States, or interstate. Provision is made for imposing other conditions necessary to prevent dissemination of plant pests in situations which are not covered by authority now contained in the present legislation. It will give similar authority to promulgate regulations governing the importation of soil, particularly surface soil, which is notorious as an avenue for the dissemination of pests.

Section 7 contains authority for the properly identified inspector to make inspections and seizures comparable to the authority contained in the Plant Quarantine Act.

Section 8 is the penalty section, and the penalties are generally parallel to those provided by the Plant Quarantine Act with this difference: the Plant Quarantine Act does not penalize violations of regulations issued under its provisions as such. The proposed act would provide penalties for violation of regulations issued under the act as well as for violations of the act itself.

Section 9 is the conventional separability section.

Section 10 modernizes the Mexican Border Act by making it possible for the inspector to supervise cleaning and treatment as well as to perform these operations himself.

Section 11 repeals the Insect Pest Act and the Mollusk Act and contains a saving clause to provide for transition of operations from the repealed basic authority to operations under the new authority.

Section 12 provides for the act to become effective upon enactment.

To summarize, the proposed Federal Plant Pest Act will give the Department of Agriculture better statutory tools with which to protect the nation's agriculture against the introduction and spread of plant pests. It will give the Department authority to take emergency action where it is not possible to take such action under existing legislation. It will clarify authority for the Department to continue to do many of the things it has been doing since the enactment of the Plant Quarantine Act in 1912. The average layman will not realize that this statute, when enacted, is on the books, although he may eventually note that he is getting better protection against pest dispersal. With respect to the movement of the material necessary for his investigations, the plant pathologist should find little difference in the restrictions which may be placed upon him under authority of law and those limitations which are now placed upon him through voluntary adherence to the policies of the Department and to the resolution of the American Phytopathological Society.

THE MOVEMENT OF PLANT PATHOGENS [1]

W. H. Wheeler [2]

The United States Department of Agriculture first undertook to surround the importation of plant pathogens with safeguards, following the suggestion of the American Phytopathological Society that such safeguards were needed.

The Society first showed interest in this problem at its 20th Annual Meeting in 1928 when it approved the following resolution offered by the Resolutions Committee: "*Resolved:* That the Society authorize appointment of a standing committee on plant quarantine and regulatory work to keep the members in touch with progress made along these lines. One of the functions of this committee would be to consider the advisability of regulating the importation of pathogenic cultures."

The following year the Committee on Quarantine and Regulatory Work made its report. One item read as follows: "At the present time there appears to be no great demand or need for regulating importation of pathogenic cultures from foreign countries."

Thereafter, the matter was not mentioned again in the Society records until the 27th Annual Meeting in 1935. At that time the organization adopted the following resolution: "*Resolved:* That in view of the potential dangers of the unrestricted entry of living cultures of plant pathogens, the Society recommends that such cultures be permitted import only on permit. At the same time the Society is cognizant of the need of living cultures for scientific purposes and recommends as free entry of such cultures as is consistent with adequate safeguards in national interests." Adopted at the same time was another resolution: "*Resolved:* That the same caution be taken with respect to the introduction of biological races of dangerous plant parasites as is considered necessary in the case of specific entities."

At the 28th Annual Meeting in 1936, the Committee on Foreign Plant Diseases and Quarantines reported that the resolutions of the 27th Annual Meeting had been presented to the Honorable Secretary of Agriculture and the chiefs of the Bureau of Plant Industry and of the Bureau of Entomology and Plant Quarantine. The Bureau of Entomology and Plant Quarantine in its response "acknowledged the inconsistency of al-

[1] Invitational paper presented December 6, 1956, at the Symposium on Foreign Plant Quarantines and Regulatory Administration at the 48th Annual Meeting of the American Phytopathological Society at Cincinnati, Ohio.

[2] Plant Quarantine Division, Agricultural Research Service, U. S. Department of Agriculture.

lowing living cultures of pathogens to enter without control from foreign countries."

Apparently the Bureau in acknowledging such an inconsistency felt obligated to do something about the Society's resolution; however, as Mr. Dean has pointed out, there was no legal basis for enforcing any regulations to govern the importation of plant pathogens as such. As the Plant Quarantine Act of 1912 was written, action could be taken against diseased plants, but the Act was silent on the quarantine status of the plant pathogens themselves.

To meet this problem, the Bureau took the only alternate course that seemed to offer promise. It endeavored to enlist the cooperation of those who wished to import cultures of microorganisms, and it found nearly all importers quite willing to assist in the matter.

From then on, saprophytic or cosmopolitan organisms encountered entering the country were considered to represent no risk and were sent on to the addressee with a letter suggesting that he should communicate with the Bureau when next he might wish to import cultures of microorganisms. By writing to report that he wished to bring in a stated number of cultures, he would make it possible for the Bureau to furnish him with official green and yellow mailing labels for use by the shipper. Such labels effect prompt delivery of the importations to the inspection station designated. At that point, the labels are removed and the parcels readdressed and returned promptly to the mail for delivery under the original postage.

When pathogens of plant quarantine importance have been encountered entering the country without the official green and yellow labels, inquiry is made of the importer to determine the purpose of the importation and the methods of safeguarding to be employed to prevent the escape and establishment of dangerous diseases. At the same time, an inquiry is addressed to the quarantine officer of the state of destination to determine his reaction to the proposed use of the pathogens by the addressee. If satisfactory information from the importer regarding the careful handling of the pathogens is received and if the state official offers no objection to such use, the importation is forwarded. At the same time, the addressee is also informed of the procedure to be followed in making future importations.

Acknowledgment must be made of the excellent cooperation received from the scientists of the nation. Rarely have any of them objected to the efforts of the former Bureau of Entomology and Plant Quarantine or the present Plant Quarantine Division to insure against the escape of serious plant pathogens imported from abroad, and rarely have any requests for cultures received unfavorable consideration. The efforts of the Plant Quarantine Division are directed primarily toward those who are not equipped to handle dangerous pathogens safely. At the same time, every effort is made to avoid delay in answering letters requesting the official mailing labels. Such correspondence receives preferred handling.

Delay in the final issuance of the mailing tags most frequently occurs when we find it necessary to communicate with the state plant quarantine officer concerned. Such references are required when the prospective importer may have requested an important pathogen or a race thereof not reported to be present in the state of destination. Then we must ask for concurrence from the state official before issuing the tags. Therefore, an importer can avoid a certain amount of delay by first referring his list of organisms to be imported to the plant quarantine officer of his own state. In doing so, it would be well to explain the need for the pathogens and the manner in which they will be safeguarded. And finally, the state official should be asked to write to the Plant Quarantine Division to give his reaction to the request.

It is quite possible that many persons do not know the name of their state plant quarantine officer and the procedure to follow in arranging for the importation of microorganisms. The list of state officers may be obtained by writing Plant Quarantine Division. A 1-page set of instructions on the importation of cultures of microorganisms is added as part of this paper.

This statement would not be complete without a reference to the state regulations governing the interstate movement of plant disease organisms. Some states do require that a person receiving such organisms shall hold a permit to do so. Permit requirements of the state in which you reside can be determined by writing to the state plant quarantine officer concerned.

In speaking of cultures, we intend to include any means whereby plant pathogens may be shipped, whether it be on agar, in host tissues, in soil, or in any other way that microorganisms as such may be transported.

INSTRUCTIONS TO CULTURE IMPORTERS

United States Department of Agriculture
Agricultural Research Service
Plant Quarantine Division
Washington 25, D. C.

To help safeguard the agriculture of the United States against the introduction or dissemination of destructive plant diseases, the Plant Quarantine Division of the U. S. Department of Agriculture cooperates with the State regulatory officials in maintaining surveillance over the importation and interstate movement of plant disease organisms. To avoid possible delay in the receipt of such material, it is suggested that prospective importers follow the procedure outlined below.

1. *Importation of nonpathogenic organisms:*
   Inform the Plant Quarantine Division in advance, at the address shown above, of the names of the cultures and the number expected, so official green and yellow mailing labels may be furnished to effect prompt delivery of the parcels.
2. *Importation of pathogenic organisms:*
   (a) Secure advance approval from the State regulatory official concerned before asking for official mailing labels. Evidence of such approval, with a list of the pathogens and a statement of safeguards to be used in their handling, should accompany the request to

the Plant Quarantine Division for mailing labels.

(b) Instruct foreign shippers to forward cultures or diseased material by either air or surface mail, using the official labels. Cultures intercepted at ports of entry without the mailing labels may be delayed in delivery since government agencies finding them will have to request instructions from the Plant Quarantine Division on their release.

(c) Redistribution of imported pathogenic organisms should be made only with the approval of the plant quarantine official of the State of destination.

(d) Those interested in human or animal pathogens should address requests for the necessary import permits as follows:

For human pathogens:

The Surgeon General
Public Health Service
U.S. Department of Health, Education, and Welfare
Washington 25, D. C.

For animal pathogens:

Biological Products Licensing Section, Animal Inspection and Quarantine Branch, ARS
U.S. Department of Agriculture
Washington 25, D. C.

THE DISCOVERY OF IMPORTANT DISEASES BEFORE THEY MOVE FROM ONE COUNTRY TO ANOTHER [1]

A. J. Riker [2]

STATEMENT OF THE PROBLEM.—The problem is well known and has long been recognized. Many of the most injurious insect pests and plant diseases have come from foreign countries.

Although the problems are similar for both entomologists and plant pathologists, comments are limited to the plant pathogens. This is intended by no means to minimize the critical and important dangers also presented by injurious insects. At the same time it is recognized that fumigation against insects is quite efficient, whereas similar treatment against fungi, bacteria, and viruses may have little effect. Furthermore, if a pathogen has invaded plant tissue but has not yet produced symptoms, it leaves an inspector helpless.

Among the serious diseases that have entered the United States are chestnut blight, potato late blight, and white pine blister rust. Many dangerous diseases occur sometimes in unexpected places and await only a suitable opportunity to enter some new country. The list of such diseases is quite long, as shown by the quarantines published by various countries. The reviews on this subject by Rex Hunt and W. A. McCubbin are well known. The extremely rapid flights of airplanes with passengers, express, and freight present complicated problems of keeping out undesirable diseases and add enormously to the difficulty for all countries.

Dangerous also are other fungi, bacteria, or viruses that may be of little or no consequence in their native habitat but that might be extremely serious in another country.

One of the important questions remaining is how to find out about these relatively little-known but dangerous disease-causing agents. This needs to be done before they are introduced into a different country and before they start dangerous epidemics on important crops.

SOME BACKGROUND CONSIDERATIONS.—Some basic ideas may be considered as fundamental to a discussion of this problem. When a new crop is introduced into a country, some of the native pathogens may attack it. The new crop may arrive through importations or through development by plant breeders. While new crops are being bred, including those bred for disease resistance, one needs also to keep in mind that various pathogens also may be either developing new races or finding new susceptible hosts. For example, oats developed for resistance to rust may be susceptible to a hitherto unknown fungus that incites seedling and culm blight.

Introduced pathogens also may attack native crops in an unsuspected way. Thus, the danger remains that a certain number may slip by in spite of the best quarantine procedures at the points of entry. An inspection of the growing crop in a foreign country before importation has been a big step forward for all concerned.

Even superlative port inspections may not provide the necessary protection. Nevertheless, they can keep out many things about which inspectors are well informed. They can at least limit the entry of foreign diseases to a minimum. These diseases then can be eradicated with suitable control measures where they appear in small areas. Furthermore, quarantines can provide time to work out and apply critical control measures, including even disease-resistant varieties.

Nevertheless, the best quarantine service is relatively helpless against exotic pathogens of unknown potential pathogenicity. Such causal agents require particular attention and study in the country where they occur. The introduction and development of the chestnut-blight fungus in the United States is a good example. It was a little-known and disregarded pathogen in its native habitat, but it produced a devastating effect when moved to a new location. In casual conversation yesterday, B. S. Crandall told of a serious unidentified rice disease in Cuba. Apparently the same or a similar disease is now causing considerable loss in Panama, Venezuela, and Japan. Reports about potentially dangerous but unknown pathogens are disturbingly fre-

[1] Invitational paper presented December 6, 1956, at the Symposium on Foreign Plant Quarantines and Regulatory Administration at the 48th Annual Meeting of the American Phytopathological Society at Cincinnati, Ohio.

[2] Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

quent.

The difficulties involved in a study of such pathogens are real and numerous. Obviously, only local progress can be made without international cooperation. The information about important plant diseases in many places over the world is quite inadequate. Even in the best informed countries, important gaps are quite apparent. There is enough truth to add barbs to the gibe that plant disease distribution maps show more the incidence of active plant pathologists than the actual distribution of the pathogens.

As already considered, some of the most serious pathogens have emerged from unexplored regions or unexpected places. Some obscure pathogens may require only removal either to a more favorable climate or to a more favorable collection of susceptible crops to display their full capabilities.

These problems are by no means new. When the speaker was President of the American Phytopathological Society in 1947, this seemed to be one of the most neglected areas in which the Society might encourage activity. In a conference arranged then by Lee Hutchins with heads of the various crop divisions in the U. S. Department of Agriculture, everyone, with a single exception, recognized the need and was enthusiastic about starting work. Each division was encouraged to present an item in the budget to finance a suitable program with the crops for which it was responsible. Then what happened was this: the budget requests were cut. In every case new items, including those for a study of foreign diseases, were dropped for the time being. Fortunately, plans have matured since then and some substantial beginnings have been made.

THE PROGRAM.—Looking to the future, the following appear to be among the more promising procedures in the study of foreign pathogens: 1) an extended study of the literature to find out what has been reported in different localities, 2) an extension of inoculations on suitable crops under conditions that are favorable for the development of diseases, and 3) plantations of selected varieties or selections of crop plants that are exposed to natural infections and that are kept under competent supervision. In connection with any procedure involving movement of seed or vegetatively propagated plants, suitable treatments, inspections, and quarantines need to be applied so as to make sure that no undesirable pathogens are moved about.

Certain important progress already has been made along these lines. Particularly pertinent is the literature review by Perly Spaulding, "Diseases of North American Forest Trees Planted Abroad." Some of these problems are of special concern to the forest pathologists because of the long time the trees live. At the same time, perhaps some of the more important tree diseases have been missed because the trees were attacked and killed before adequate observations and records were made of the causal agents. For these and other crops, much would be gained if John A. Stevenson's "Foreign Plant Diseases" could be brought up-to-date and printed.

Inoculations with important pathogens is one of the pathologist's most important tools while developing disease-resistant varieties; however, except rarely under rigidly controlled conditions, he is limited to the pathogens already present in his region. This applies also to unusually virulent or new races. Ordinarily he dare not import pathogens or new races of them even for greenhouse trials and certainly not for field trials. For tests against foreign pathogens, the crop needs to be grown where the pathogen is active.

In any planting program, the work will succeed only with satisfactory international cooperation in which the cooperating countries both benefit. It should start on a small scale, so that the inevitable mistakes can be made and corrected before they become problems. Many obstacles appear to the successful prosecution of this kind of a program. One of the greatest difficulties is to achieve mutual understanding. Fortunately, all of the obstacles have been overcome in certain important cases.

For example, outstanding success has been accomplished under the capable leadership of the Field Crops Research Division of the U. S. Department of Agriculture. As many of you know, scientists have had nurseries of wheat for some years in North, Central, and South America and more recently have had plantations of barley and oats. Suitable selections from the world collection of cereal varieties have been planted and have been exposed to natural inoculation by whatever pathogen was present. The results have been so outstanding that nurseries have been planted elsewhere. Through the cooperation of Departments of Agriculture, colleges and experiment stations of many nations and states, the Rockefeller Foundation, United Nations Food and Agriculture Organization, and the International Cooperation Administration, world collections of about 15,000 wheats, 8000 barleys, and 4000 oats have been screened for sources of resistance to rusts. For 1957, there will be 53 nurseries in 25 countries. The possibilities for rice, beans, potatoes, and other crops are obvious. Apparently, some work has been started with such crops.

Although the technical benefits have been enormous, even greater dividends have come through an exchange of seed, ideas, and techniques between different countries, with corresponding improvement of international cooperation and understanding.

The best auspices for doing this work could and no doubt would vary from country to country, from crop to crop, and possibly from pathogen to pathogen. In the above example, different kinds of cooperation contributed to its success. No reason is apparent why such work should be the exclusive activity of any one agency. On the contrary, various advantages appear for different ones doing that portion of the work for which they are best qualified.

In any case, international understanding, cooperation, and mutual benefit, as well as skillful, technical procedures, are essential for success.

THE EFFECT OF VIRUS INFECTION ON YIELD AND CHEMICAL COMPOSITION OF LADINO CLOVER [1]

K. W. Kreitlow, O. J. Hunt, and H. L. Wilkins [2]

SUMMARY

Healthy and virus-infected clonal lines of Ladino white clover that were genetically identical were compared for yield and chemical composition. Healthy plants, verified as virus free by tests on Bountiful bean, were cloned into 2 parts. One clonal line was retained as healthy, the other was inoculated with a mixture of alfalfa and bean yellow mosaic viruses. In greenhouse tests, 320 healthy and 320 virus-infected clonal plants were compared in a compartmented nutrient-culture bench containing sand. Identical clonal material was compared in replicated field plots. Virus infection in both greenhouse and field tests reduced yielding ability of the clones from 23 to 55 per cent. Virus infection stunted most clonal lines tested and resulted in less lateral spread than normal. Yielding ability of a clone that showed practically no visible symptoms of virus infection was reduced as much as that of clones that exhibited severe virus symptoms. Chemical analyses indicated that healthy clonal material was higher in percentage of crude fiber than was the infected, whereas the virus-infected forage was higher in nitrogen-free extract than was the healthy. Stage of maturity at which forage was harvested had a greater influence on percentage protein than did virus infection.

Ladino white clover, (*Trifolium repens* L.), is one of the most important forage legumes. It is attacked by plant diseases that reduce potential yield, affect quality, and decrease persistence. Unlike cash crops such as wheat or potatoes, where yield is measured in terms of bushels per acre at the end of the growing season, forage crops such as Ladino clover are harvested or grazed several times a season, usually for a period of several years. Consequently, damage from plant diseases is difficult to assess and usually is not apparent until the stand deteriorates or is lost.

Many fields of Ladino clover thrive for 2 or 3 years, then the productivity declines, and the clover nearly disappears. Examination of such fields often reveals a progressively higher percentage of plants infected by virus diseases as the stand ages. Viruses infecting Ladino clover in the field have been described by several authors (2,5). It has been suggested (5,6) that affected plants become weakened and are more susceptible than are healthy plants to adverse conditions such as drought and winter injury. Similar observations have been made for red clover (4). Roberts (8) recently substantiated such observations with experimental data. In his tests, Ladino clover plants artificially inoculated with alfalfa mosaic virus failed to survive low-temperature treatments. Comparable healthy plants were injured, but most of them survived. Complaints by farmers concerning loss of stands and low yields from Ladino clover infected by viruses have been reported (1). Aside from observations made by Houston and Oswald (2) and Hutton and Peake (3), only limited data on how much yielding ability of clovers is reduced by virus infection are available. In their tests, Houston and Oswald (2) inoculated groups of plants of Ladino clover growing in the greenhouse with single virus strains and with mixtures of alfalfa mosaic and bean yellow mosaic virus strains. Their results indicated that strains of alfalfa mosaic virus caused the most severe stunting, with yields of infected plants about 2/3 that of the checks. Infection by a mixture of alfalfa mosaic and bean yellow mosaic viruses resulted in yields of less than 1/2 that of the checks. Reductions in yield ranging from 26.1 to 76.7 per cent in mean weight of artificially inoculated plants of subterranean clover (*Trifolium subterraneum* L.) infected with bean yellow mosaic virus were reported by Hutton and Peake (3) in tests made in Australia.

In order to evaluate critically the effect of virus infection on Ladino clover, a technique was used at Beltsville, Maryland, in which clonal material of virus-infected and healthy Ladino clover was compared (7). The comparisons were made both in the greenhouse and in the field with respect to rate of growth, yield, and chemical composition of plants. The results obtained suggest that virus infection in Ladino clover causes considerably more damage than is generally realized.

MATERIALS AND METHODS.—Clonal lines of healthy and virus-infected Ladino clover were obtained in the following manner. A healthy Ladino clover plant, verified as free of viruses by rubbing juice from its leaves onto the first leaves of a Bountiful bean plant, was cloned into 2 parts. One clonal line was retained as "healthy," and the other was artificially inoculated with virus. Both lines, derived originally from a single plant, were then cloned independently and kept well separated until a sufficient number of healthy and virus-infected plants was obtained. Since it is known that Ladino clover in the field is commonly infected simultaneously by a mixture of alfalfa mosaic and bean yellow mosaic viruses, a culture comprising these 2 viruses, maintained in an infected Ladino clover clone, was used in all inoculations. Virus was intro-

[1] Accepted for publication January 29, 1957.

[2] Research Pathologist, Research Agronomist, and Chemist, respectively, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

duced into healthy rooted clonal cuttings by rubbing a gauze pad dipped in virus juice over the surface of leaves dusted with No. 400 Carborundum powder. The healthy and virus-infected rooted cuttings were transplanted into separate partitioned compartments of a nutrient-culture bench in a greenhouse. The bench contained sand and was divided into 20 compartments, each 18 × 28 in. and 8 in. deep. Each compartment was filled with washed 8-mesh quartz sand. For each experiment in the greenhouse, 32 healthy or virus-infected rooted cuttings spaced approximately 4 in. apart were transplanted into each of the 20 compartments, thus 320 healthy and 320 virus-infected clonal cuttings from 1 or several lines of Ladino clover could be compared. Plants in each compartment of the greenhouse bench were kept from touching each other by a barrier of wire glass 12 in. high. They were fed independently with a complete nutrient solution of the following composition: 112 ppm N as $Ca(NO_3)_2 \cdot 4H_2O$ and NH_4NO_3; 46 ppm P as $Ca(H_2PO_4)_2 \cdot H_2O$ and $NaH_2PO_4 \cdot H_2O$; 110 ppm K as K_2SO_4 and KCl; 100 ppm Ca as $Ca(NO_3)_2$ and $Ca(H_2PO_4)_2 \cdot H_2O$; 24 ppm Mg as $MgSO_4$ and $MgCl_2 \cdot 6H_2O$; 0.15 ppm Zn as $ZnSO_4 \cdot 7H_2O$; 0.06 ppm Cu as $CuSO_4 \cdot 5H_2O$; 0.5 ppm B as H_3BO_3; 2 ppm Fe as $FeSO_4 \cdot 7H_2O$; 1 ppm Mn as $MnSO_4 \cdot H_2O$.

The nutrient solution, held in separate 5-gal. bottles for each compartment, was forced upward through the sand to the surface 2 or 3 times each day by means of an automatically controlled air pressure system. When the air pressure was released, the solution drained by gravity into the 5-gal. containers. Fresh nutrient solution was supplied every 2 weeks. When the plants reached the grazing stage of growth they were clipped, the forage dried, and dry weight of the harvested material recorded. In the harvesting, all healthy plants were clipped first to avoid contamination with virus.

Since greenhouse tests are not always indicative of what may happen in the field under natural conditions, a similar experiment was conducted in field plots. Healthy and virus-infected rooted clonal cuttings identical with those used in the greenhouse tests were transplanted to field plots on April 29, 1955, and May 22, 1956. Each healthy and virus-infected clonal line was established in triplicate in 3 × 10-ft. plots, each containing 120 plants spaced approximately 6 in. apart. Treatments in each field test were completely randomized and replicated in triplicate. In the field test conducted in 1955, a 3-ft. space was left between plots to reduce possible contamination. A 6-ft. space was left between plots in the 1956 field test, since earlier experience showed that lateral growth of healthy plants spread across the 3-ft. space in 1 growing season. Forage from an entire 3 × 10-ft. plot was harvested and weighed the first time a plot was cut following establishment. In order to observe comparative growth of healthy and virus-infected plants, lateral growth of stolons was not restricted other than to keep the plots reasonably compact. Consequently, plots with healthy plants enlarged by lateral growth more rapidly than did those with virus-infected plants. To compare yields in subsequent cuttings, a uniform 3 × 10-ft. swath of forage was harvested from each plot unless indicated otherwise. The remainder of the plot was then clipped to the same height. All healthy plots were harvested first to avoid contaminating them with virus. The forage obtained was dried at 160–180°F, and the yield was converted to pounds of dry matter per acre. To minimize contamination of healthy plants by viruliferous insects, all plots were sprayed at least once a week and after every rain.

Chemical analyses were made according to the procedures of the Association of Official Agricultural Chemists (9), substantially as given in the several appropriate sections.

RESULTS.—*Greenhouse tests.*—In the first greenhouse test conducted in 1955, 32 healthy rooted cuttings of a single clone of Ladino clover, R-63, were transplanted into each of 10 alternate compartments in the nutrient-culture bench, and an equal number of virus-infected rooted cuttings were transplanted into each of the 10 other compartments. Thus, 320 healthy and 320 virus-infected clonal cuttings of a genetically identical line were compared. Within 2 weeks, striking differences in rate of growth were evident between healthy and virus-infected plants. These differences persisted and became even more apparent as the plants matured (Fig. 1, A). Two harvests, one on March 30 (34 days following transplanting) and one on April 20 (21 days after the first harvest), were obtained from this material. The average yield of dry matter from the 10 replications of healthy clover in the first harvest was 35.3 g, and that from virus-infected clover was 15.8 g. In the second harvest, the yields were 38.7 and 22.6 g, respectively. The percentage yield reduction from virus infection was 55.2 in the first harvest and 41.6 in the second. The greater difference in yield in the first harvest than in the second was probably due to slow recovery following transplanting of virus-infected plants. The over-all percentage yield reduction for both harvests was 48.4. The striking effect of virus infection on yield was confirmed by statistical analysis, which showed the differences to be significant at the 1 per cent level.

A more extensive test was conducted in the greenhouse during the winter of 1956. In this test, 5 virus-infected clones and their healthy counterparts were compared. These were selected to represent germ plasm more characteristic of a variety than would be the case with a single clone. The clones exhibited gradations in symptoms from severe to hardly noticeable. One of the clones, R-63, was tested in the greenhouse and in the field in 1955. Clone R-92 also was tested in the field in 1955. The other 3 clones had not been tested previously. Since the number of clones was larger in this test than in previous ones, it was necessary to reduce the number of replications to 2. Nevertheless, the data supported those obtained earlier and indicated in addition that damage from virus

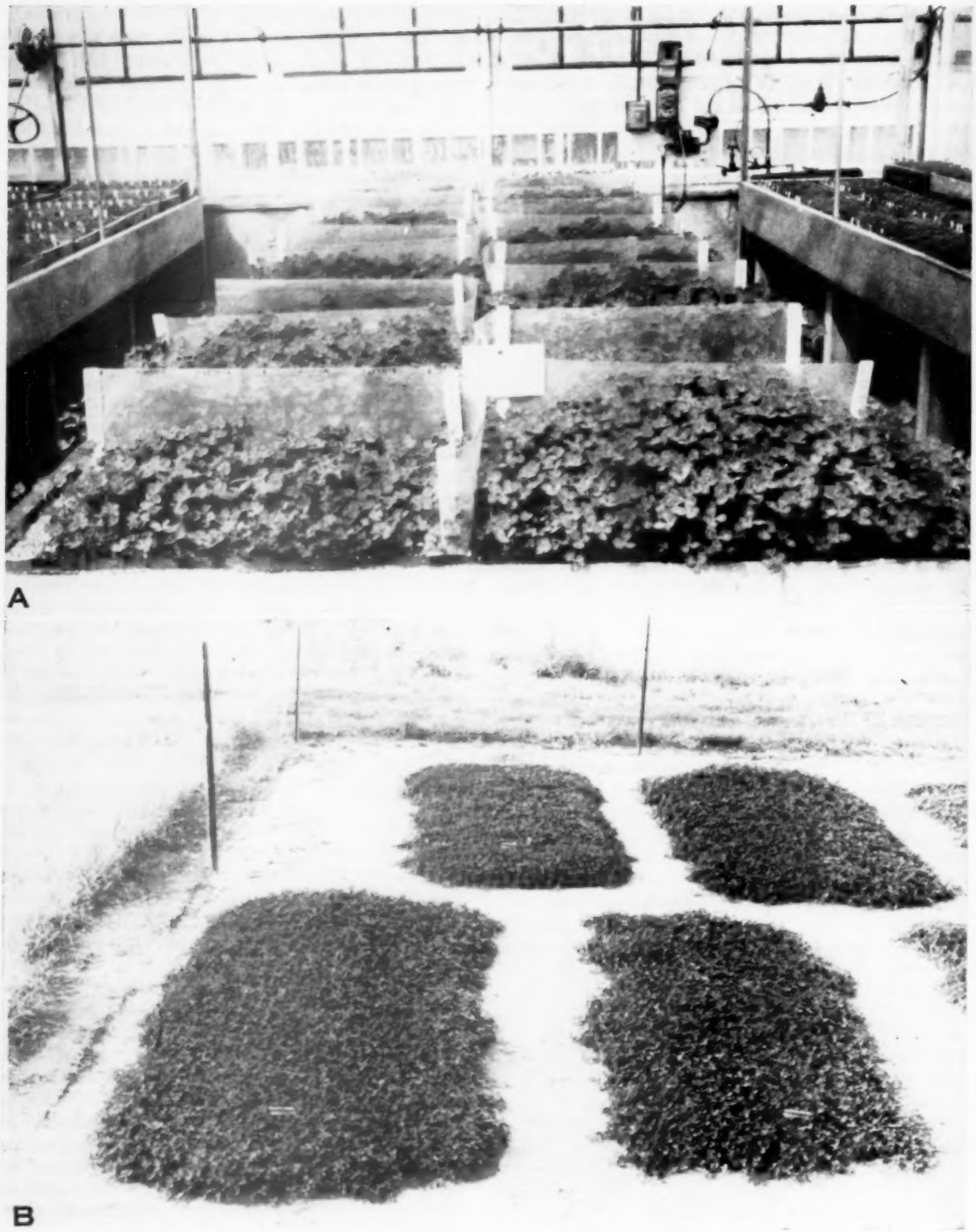


FIG. 1. Comparative growth of healthy and virus-infected (alfalfa mosaic and bean yellow mosaic viruses) clones of Ladino clover. A) Alternate blocks of clone R-63: healthy plants in right foreground and virus-infected plants in left foreground. B) Field plots of clone R-92: healthy plants in left foreground and virus-infected plants in right foreground.

infection probably occurs in Ladino white clover plants from many different sources. This was somewhat unexpected, since visual examination of clone R-92 indicated little damage from virus infection. Both healthy and virus-infected plants grew tall and were large-leafed. Leaves of virus-infected plants were not deformed or conspicuously mottled. When plants were harvested and the growth examined carefully, it was evident that healthy plants of clone R-92 produced much denser growth than did virus-infected plants. Although there was no difference in height of the 2 kinds of plants, there were appreciably fewer petioles produced on infected plants than on healthy ones. A summary of the data obtained is presented in Table 1. Percentage yield reductions for 3 cuttings obtained in the greenhouse test ranged from 36.2 for clone R-63 to 48.1 for clone R-92. Like the earlier greenhouse results, these also were highly significant when analyzed statistically.

Field tests.—The striking differences obtained in the greenhouse were substantiated in a 1955 field test in which healthy and virus-infected material of 2 different clones, R-63 and R-92, were used. Within a few weeks after transplanting, differences in the comparative recovery and lateral spread among healthy and virus-infected plants were readily noticeable. Stunting among virus-infected plants of clone R-63 was obvious, and it became even more pronounced as the plants grew larger and approached the stage for harvesting (Fig. 1, B). The average yield and percentage yield reduction for 2 harvests of this material are shown in Table 2. The first cutting was made July 13, 75 days following transplanting to the field. Weather conditions during much of this period were hot and dry, resulting in poor growth. The yield of each virus-infected clone in the first harvest was less than 50 per cent of that of the corresponding healthy clone. The second harvest was made September 2, 51 days following the first cutting. The percentage yield reduction for virus-infected clone R-92 was comparable to that obtained in the first harvest. Yield of dry matter of virus-infected clone R-63, however, was substantially greater than that in the first harvest. This resulted in a reduction in potential yield of only 26.2 per cent compared with 54.3 per cent in the first harvest. The discrepancy apparently was due to excessive development of weeds in the plots of the virus-infected clone; these contributed to the weight of dry matter. No comparable development of weeds occurred in the healthy plots or in plots of virus-infected clone R-92. Statistical analysis showed results to be significant at the 1 per cent level.

TABLE 2.—*Comparative average yield from 2 harvests of healthy and virus-infected plants of 2 clones of Ladino clover grown in the field in 1955*

Clone	Harvest	Lb. dry matter/acre [a]		Percentage yield reduction
		Healthy	Infected [b]	
R-63	1	943	431	54.3
	2	829	612	26.2
	1 + 2			40.2
R-92	1	2267	1073	52.7
	2	908	408	55.1
	1 + 2			53.9

[a] Each figure is the average from 3 replications of 3 × 10-ft. plots.
[b] Plants inoculated with a mixture of alfalfa mosaic and bean yellow mosaic viruses.

A second set of plots was established May 22, 1956, with the 5 clones of Ladino clover used in the 1956 greenhouse test. Two of the clones, R-63 and R-92, were identical with the clones used in the 1955 field test. Three harvests were made from these plots during the 1956 growing season. In the first harvest, July 18, the entire plot (approximately 3-ft. wide) was cut and the dry-matter yield obtained. Since the plots had enlarged by lateral growth, a 36-in. swath was taken from the center of each plot for the second harvest on August 17. The third harvest, September 10, was done by clipping with a 27-in. lawnmower to remove as many seed heads as possible and to prevent reseeding of the plots. Yields from each cutting were adjusted to comparable levels for comparison. Virus infection reduced potential yield of the clones 23.2–55.8 per cent (Table 3). These results compare favorably with those obtained for the 5 clones in the greenhouse test and with the earlier field test. They emphasize the substantial reduction in yielding ability that can be expected to occur among plants in a population that is infected with these viruses.

Chemical composition.—Since striking differences in yield were obtained between healthy and virus-infected

TABLE 1.—*Comparative average yield of dry matter from 3 harvests of healthy and virus-infected plants of 5 clones of Ladino clover grown in the greenhouse*

Clone	Yield in grams [a]		Percentage reduction in yield from virus infection
	Healthy	Infected [b]	
25	33.4	20.0	40.2
32	21.4	12.9	39.8
42	27.3	16.1	41.1
R-63	18.0	11.5	36.2
R-92	25.8	13.4	48.1

[a] Each figure is the average of 10 replications, each consisting of 32 plants.
[b] Plants inoculated with a mixture of alfalfa mosaic and bean yellow mosaic viruses.

TABLE 3.—*Comparative average yield from 3 harvests of healthy and virus-infected plants of 5 clones of Ladino clover in the field (1956)*

Clone	Lb. dry matter/acre [a]		Percentage reduction in yield from virus infection
	Healthy	Infected [b]	
25	2600	1149	55.8
32	1796	1101	38.7
42	2634	1393	47.1
R-63	2649	1515	42.8
R-92	2678	2057	23.2

[a] Each figure is the average from 3 replications of 3 × 10-ft. plots.
[b] Plants inoculated with a mixture of alfalfa mosaic and bean yellow mosaic viruses.

TABLE 4.—*Chemical composition of healthy and virus-infected plants of 2 clones of Ladino clover* [a]

Date of harvest and analysis made	Clone R-63		Clone R-92	
	Healthy	Infected	Healthy	Infected
July 13, 1955				
Ash	11.70	11.84	14.55	13.10
Crude fiber	20.39	17.16	20.68	19.20
Crude protein	20.45	23.40	19.83	20.15
Ether extract	2.60	2.47	2.61	2.64
Nitrogen-free extract	44.85	45.14	42.33	44.90
September 2, 1955				
Ash	11.35	12.43	12.31	14.92
Crude fiber	14.88	14.49	17.24	15.40
Crude protein	27.28	26.60	26.99	24.76
Ether extract	3.35	2.75	2.95	2.21
Nitrogen-free extract	43.14	43.73	40.52	42.72

[a] Percentage by weight of oven-dry material. Both healthy and infected (by alfalfa mosaic and bean yellow mosaic viruses) plants were grown in field plots.

Ladino clover, samples harvested from both greenhouse and field plots were analyzed chemically to determine whether or not virus infection influenced chemical composition. Analysis of healthy and virus-infected clone R-63 grown in the greenhouse in 1955 indicated that the healthy clonal material was higher than the infected in ash, crude fiber, and ether extract, whereas the virus-infected clonal material was higher than the healthy in crude protein and nitrogen-free extract. To investigate these differences further, analyses were conducted on forage harvested from healthy and virus-infected plants from field plots of clones R-63 and R-92 established in 1955. Separate analyses were conducted on forage harvested on July 13 and September 2. The only consistent differences were small changes in percentage of crude fiber and of nitrogen-free extract (Table 4). The percentage of crude fiber was higher in healthy than in infected plants of each clone for both cutting dates whereas the percentage of nitrogen-free extract was higher in virus-infected than in healthy plants. This suggests that the virus-infected tissue was less mature than the healthy material. Virus infection causes many plants to persist in a vegetative state rather than reaching maturity.

Among the other analyses, percentage of protein was higher in virus-infected than in healthy forage of both clones in the July 13 harvest, whereas the reverse was true in material harvested September 2. These results suggest that environmental conditions influenced the percentage of protein more than did virus infection. As suggested by Roberts (8), virus infection in Ladino clover may interfere with carbohydrate synthesis and translocation. This would influence persistence of virus-infected plants during periods of environmental stress. The information obtained to date on chemical composition suggests that further investigation of the nitrogen-free extract might be of interest.

DISCUSSION.—From the greenhouse and field data presented, it is apparent that virus infection can reduce the yielding ability of Ladino clover from 23 to 55 per cent. The evaluation of healthy and virus-infected clonal material presented in these tests compares favorably with the yield data published by Houston and Oswald (2) for virus-infected groups of plants of Ladino clover growing in the greenhouse and with data presented by Hutton and Peake (3) in Australia for plants of subterranean clover artificially inoculated with bean yellow mosaic virus. Although there may be a differential response among clones of Ladino clover to amount of damage from virus infection, it is evident that all clones tested were affected to a substantial degree. Field observations previously have indicated that when $\frac{1}{3}$ to $\frac{1}{2}$ of the plants in a field of Ladino clover were estimated to be infected with virus, the forage yield from the field was appreciably less than that from healthy plants. Additional field tests are underway to determine the effect of virus infection on persistence and on yield in subsequent seasons. More detailed chemical analyses also are necessary to determine the effect of virus infection on various chemical constituents. This in turn may give some clues concerning possible nutritive value of the respective healthy and virus-infected material. The widespread occurrence of virus infection among plants in fields of Ladino clover and the substantial reduction in yielding ability that results stress the need for obtaining resistant plants.

FIELD CROPS RESEARCH BRANCH
AGRICULTURAL RESEARCH SERVICE
U.S. DEPARTMENT OF AGRICULTURE
BELTSVILLE, MARYLAND

LITERATURE CITED

1. FENNE, S. B. 1952. Severe virus disease on Ladino clover in Virginia. Plant Disease Reptr. **36**: 491.
2. HOUSTON, B. R., AND J. W. OSWALD. 1953. The mosaic virus disease complex of Ladino clover. Phytopathology **43**:271-276.
3. HUTTON, E. M., AND J. W. PEAKE. 1954. Varietal reactions of Trifolium subterraneum L. to Phaseolus virus 2 Pierce. Australian J. Agr. Research **5**: 598-607.
4. KING, T. H., M. F. KERNKAMP, T. P. REILING, AND N. OSHIMA. 1955. We're fighting virus diseases of legumes. Minn. Farm and Home Sci. **13**: 6-7. October.
5. KREITLOW, K. W., AND W. C. PRICE. 1949. A new virus disease of Ladino clover. Phytopathology **39**: 517-528.
6. KREITLOW, K. W. 1955. Virus diseases of grassland crops. Plant Disease Reptr. **39**: 343.
7. KREITLOW, K. W., O. J. HUNT, AND H. L. WILKINS. 1956. The effect of virus infection on yield, flowering, and chemical composition of Ladino clover. (Abs.) Phytopathology **46**: 17.
8. ROBERTS, D. A. 1956. Influence of alfalfa mosaic virus infection upon winter hardiness of Ladino clover. (Abs.) Phytopathology **46**: 24.
9. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1950. Official methods of analysis. Ed. 7. Washington. 900 p.

TEMPERATURE AND SEASONAL EFFECTS ON SEEDLING REACTIONS OF BARLEY VARIETIES TO THREE RACES OF PUCCINIA GRAMINIS F. SP. TRITICI[1]

F. L. Patterson, R. G. Shands, and J. G. Dickson [2]

SUMMARY

The seedling reactions of 52 barley varieties to 3 races of *Puccinia graminis* f. sp. *tritici* were determined at temperatures of 16°, 20°, 24°, and 28° C during and following inoculation. Reactions often were not clearcut but extended over a considerable range. A combination of number and type of pustules was the best measure of differences among varieties. Temperature influenced the frequency of pustules, pustule size, and reaction type. Seasonal changes in reactions also occurred. Differences among races were small.

For most varieties and hybrids, there was close agreement between seedling reactions at 28° C and the adult-plant reactions in field or greenhouse.

Seedling leaves are inoculated to determine the reaction of varieties of wheat and oats to specific races of varieties of *Puccinia graminis* Pers. Good agreement, in many cases, between seedling reactions in the greenhouse and adult-plant reactions in the field has made seedling tests a valuable technique in breeding for resistance to stem rust.

Although sometimes influenced by environmental factors, seedling reactions of wheat and oats are generally distinct. Seedling reactions of barley to different races of *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. have not been so distinct.

This paper reports seedling and adult-plant reactions of barley (*Hordeum vulgare* L.) varieties to races 17, 19, and 56 of *P. graminis* f. sp. *tritici* under varied environmental conditions and results of limited comparisons of seedling reactions to inoculations with 2 of these races and with 1 isolate of *Puccinia graminis* f. sp. *secalis* Eriks. & E. Henn.

REVIEW OF LITERATURE.—Waterhouse (8) reported that seedling reactions of barley varieties to *P. graminis* f. sp. *tritici* were not so clearcut as were reactions to *Puccinia anomala* Rostr. Varieties quite susceptible to stem rust showed considerable chlorosis. Pustule size was the chief feature distinguishing between resistance and susceptibility.

Johnson and Buchannon (3) found that the reactions of seedling leaves and of adult-plant stems of barley to *P. graminis* were not so closely related as they were in wheat or oats. Seedling tests of barley to *P. graminis* f. sp. *tritici* were of more value than were tests to *P. graminis* f. sp. *secalis*. With the rye stem rust, the seedling leaves of the different barley varieties reacted much alike and in general indicated a moderate degree of resistance, but the same varieties were susceptible in the adult-plant stage in the field. A range in seedling reaction types on the same leaf was common. The months of the year and the greenhouse temperatures during these tests were not stated.

Miller and Lambert (5) studied the seedling reactions of 250 barley varieties to race 15B of *P. graminis* f. sp. *tritici* at relatively low (70°F) and relatively high (82°F) temperatures. They found that there was not sufficient similarity between the reactions on barley seedlings and those usually occurring on wheat to permit the complete adoption of the standard reaction types as described by Stakman and co-workers (7). Miller and Lambert found a wide range of reaction types on the same leaf and considerable chlorosis around large pustules on barley. Reaction types were based primarily on pustule size. Although the tendency was for greater susceptibility at the higher temperature, the differentiation between susceptibility and resistance was better. A statistically significant but not perfect agreement between seedling reactions and adult-plant reactions in the field was obtained.

Andrews (2) found a temperature of 80°–85° satisfactory and one of 65°–70° unsatisfactory for classifying barley seedling reactions to race 15B of *P. graminis* f. sp. *tritici*.

Whereas Waterhouse (8) noted that a 2 reaction in winter may increase to a 2+ or 2++ in summer, he did not state whether or not it was a seasonal or a temperature effect.

Differential varietal reactions to certain races and biotypes of *P. graminis* f. sp. *tritici* in the seedling stage have been reported. Differential reactions to biotypes of race 59 were obtained with Mars, Moore, Plains, Peatland, and other varieties possessing the genetic factor *TT* for resistance (1). Differential seedling reactions of other barley varieties to races 34, 43, and 45 were reported by Waterhouse (8). All 8 possible combinations of resistance and susceptibility to the 3 races were found in the varieties tested. The relationship of the resistance of these varieties to the genetic factor *Tt* was not reported.

MATERIALS AND METHODS.—Seedling reactions of 52 barley varieties or lines to races 17, 19, and 56 of *P. graminis* f. sp. *tritici* were obtained at controlled green-

[1] Accepted for publication January 27, 1957.
Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Supported in part by funds supplied by the Wisconsin Alumni Research Foundation.

[2] Formerly Graduate Assistant, Department of Agronomy, University of Wisconsin, now Professor of Agronomy, Purdue University; Professor of Agronomy, University of Wisconsin and Agronomist, U. S. Department of Agriculture; Professor of Plant Pathology, University of Wisconsin, and Agent, U. S. Department of Agriculture, respectively.

house air temperatures of 16°, 20°, 24°, and 28° C. Six separate inoculation series extending from December to March were used to test the 52 varieties. Seedling reactions of the same 52 varieties to race 17 at 16°, 20°, 24°, and 28° C were obtained in April and in October to compare reactions at different seasons of the year.

Adult-plant reactions for these same varieties were obtained in single-race and composite-race isolation nurseries of races 17, 19, and 56 in the field at Madison, Wisconsin, in each of 2 years.

Forty-six of these varieties previously classified as resistant in the field included 1) the commercial varieties Kindred (C.I. 6969),[3] Plains (C.I. 7250), Moore (C.I. 7251), and Peatland (C.I. 5267); 2) breeding stocks Chevron (C.I. 1111), Swiss Spring (C.I. 7025), and Valentine (C.I. 7242); and 3) 39 selections from the World Barley Collection originating from stem rust resistant plant selections in the United States from farmers' fields or from commercial lots, C.I. 6606 to C.I. 6615, inclusive, C.I. 7124, C.I. 7125, and C.I. 7158 to C.I. 7184, inclusive. Four commercial varieties, Trebi (C.I. 936), Oderbrucker (C.I. 4666), Spartan (C.I. 5027), and Tregal (C.I. 6359), and 2 breeding stocks, Rabat (C.I. 4979) and Brachytic (C.I. 6572) were chosen for their susceptibility under field conditions.

In all seedling tests, about 10 seedlings of each of 2 varieties were grown in natural light in 4-in. pots divided by a wooden pot label. A soil composed of 3 parts of compost to 1 part of sand was used without additional fertilizer. The seedlings were grown at temperatures averaging about 65° F previous to inoculation. The resistant variety Valentine and the susceptible variety Rabat were included as checks in each test series. Two replications inoculated at the same time were used in each test.

Seedlings were inoculated when the first leaves were fully developed and the second leaves 1–2 in. long. The bloom was removed from the leaves by rubbing them gently with moistened fingers. Seedlings of all varieties to be inoculated with a given race were inoculated at one time as uniformly as possible by brushing the moistened leaves with seedlings bearing inoculum. The seedlings were then placed for 48 hours in moist chambers in temperature houses maintained at 16°, 20°, 24°, and 28° C. High humidity was maintained in the moist chambers by mist from atomizers. Air temperatures within the moist chambers were often 1°–2° below the temperatures of the houses because of the cooling effect of evaporation. Inoculations were made with pure cultures of races 17, 19, and 56 of *P. graminis* f. sp. *tritici* maintained on seedlings of the susceptible barley variety Rabat. Original cultures of the 3 races were obtained from the Federal Rust Laboratory, St. Paul, Minnesota.[4]

[3] Accession number Cereal Crops Section, Agricultural Research Service, U. S. Department of Agriculture.

[4] Inoculum was furnished by E. C. Stakman and W. Q. Loegering.

Seedlings were removed from moist chambers after inoculation and placed on benches in the same temperature house until rust reactions were fully differentiated. Readings of infection types were based on the scale described by Stakman and co-workers (7) for stem rust of wheat, and the number of pustules of each type was recorded.

Deviations from these general methods were necessary in some studies of the influence of preinoculation temperature, of the influence of day length and light intensity on seedling reaction type, of the reactions to isolates within races, of the reactions of F_2 seedlings, and of the relation of seedling to adult-plant reactions.

EXPERIMENTAL RESULTS.—*Seedling reaction types.*—The seedling-reaction types on barley were not so distinct as those observed on wheat, and they frequently extended over a range more limited than typical X reactions. Chlorosis was general on varieties that developed large pustules. No completely susceptible reaction was obtained on any barley variety tested. The most highly resistant varieties gave zero or flecking reactions, and sometimes a few pustules developed. Differences among varieties on which both flecking and pustules developed were best indicated by the number and type of pustules. Data were taken on pustule type and on number of pustules; these were expressed as fractions with type as the numerator and average number of pustules per 10 leaves as the denominator. Rabat was included in each inoculation series as an indicator of the level of infection.

Green-island reactions surrounding moderately small to moderately large pustules were frequent. A semichlorotic, seminecrotic area with a sharply defined margin and a moderately small pustule was interpreted as a deviation from the green-island reaction. Relatively small pustules also occurred in chlorotic areas. These were the typical low-temperature reactions of varieties susceptible in the adult-plant stage. Typical 1 reactions occurred but were not frequent. A variety expressing both flecks and pustules usually gave island reactions or moderate-sized pustules in chlorotic areas; it seldom gave pustules in necrotic areas.

Effect of temperature on severity of infection and incubation period.—The influence of temperature on severity of infection was determined for the variety Rabat, the susceptible check in stem rust studies. An analysis of variance was made of the number of pustules on Rabat in the 6 series of inoculations involving races 17, 19, and 56 of *P. graminis* f. sp. *tritici* at 16°, 20°, 24°, and 28° C. The interactions of temperature × series, races × series, and temperature × races × series were used as estimates of error. Differences between temperatures, and between series were statistically significant at the 1 per cent level. Differences between races and between duplicates and the interaction of temperature × races were not statistically significant. The mean number of pustules for 10 seedling leaves was 41.2 at 16°, 41.5 at 20°, 73.0 at 24°, and 95.4 at 28° C (standard error = 7.7).

Limited experiments with seedlings inoculated and held at 30° C indicated that this temperature was unfavorable both for barley and for stem rust development. Severity of infection on Rabat was lower at 30° than at 28° or 24°.

The incubation period was longest at 16° and became progressively shorter at 20°, 24°, and 28° in all inoculation series, with averages of 12.2, 9.5, 7.8, and 6.7 days, respectively. In limited experiments at 30°, the incubation time was the same as at 28°. Races responded similarly with reference to incubation period.

Effects of temperature and season on reaction types.—The Valentine variety, included in each inoculation series as the resistant check, was one of the most highly resistant varieties tested. The Rabat variety, included in all tests as the susceptible check, was one of the most susceptible varieties tested. Valentine was highly resistant and Rabat was susceptible in the adult-plant stage in the field.

The greenhouse temperature during the following inoculation of seedlings influenced both reaction type and pustule size. In general, for varieties susceptible in the adult-plant stage in the field, pustule size on seedlings was greatest at 28° and progressively less at 24°, 20°, and 16° C. Within a variety, the range in host reaction types was usually least at 28° and increased with decreasing temperatures. For most varieties resistant in the field, the highest seedling resistance was expressed at the higher temperatures by reduced size and number of pustules. Differences between resistance and susceptibility were usually most clearly expressed at 24° and 28°.

Since the reactions during the winter months differed from those in spring or fall, descriptions of the reactions of resistant and susceptible varieties at 16°, 20°, 24°, and 28° C are given for winter and for spring. Seedling reactions of Valentine and Rabat in winter at the different temperatures are illustrated in Figure 1. The typical winter reactions for the susceptible Rabat variety were moderate or moderately large pustules in slightly chlorotic areas at 24° and 28°, moderately small pustules in green islands at 20°, and small to moderate pustules in slightly chlorotic areas at 16°. The typical reactions in winter for Valentine, a highly resistant variety, were zero or zero-flecking at 28° and 24° and zero and zero-flecking reactions plus some small pustules in chlorotic or green islands at 20° and 16°. The resistant and susceptible varieties could be differentiated easily at 28° or 24° but not at 20° or 16°.

Limited experiments with different day temperatures and low night temperature indicated that the reaction types were similar to those at a continuous temperature of the average of day and night temperatures.

In spring or fall, the reactions of both resistant and susceptible varieties tended toward greater resistance than in winter. The pustule size on Rabat was less in spring than in other seasons, and green-island reactions were common at 24° and 28° C. Chlorosis without islands was common at 20° and chlorosis and necrosis common at 16°. These reactions resembled those obtained at 16° and 20° in winter.

For the resistant Valentine variety, the zero-flecking reaction was much more distinct at all temperatures in spring or fall tests than in the winter. The occurrence of small pustules along with zero-flecking was much less frequent in spring and fall tests than in winter tests. Typical reactions of Valentine and Rabat in the spring are shown in Figure 2. Winter and spring reactions are compared in Table 1.

Although the pustule size of susceptible varieties was smaller in spring and fall than in other seasons, these varieties were clearly distinguishable from resistant varieties at 24° and at 28° C. The barley seedlings that developed at 24° and 28° in the spring were more sturdy than those that developed in the winter because of the greater amount of sunlight in the spring.

The influence of preinoculation temperature on reaction types was studied in 2 tests. Plants grown in the temperature houses at 16°, 20°, 24°, or 28° C prior to inoculation were inoculated with race 56 and held at the same temperatures until reactions developed. In 1 series, all were planted at 1 time and inoculated at the appropriate stage. In the other series, the plantings were timed so that all seedlings were ready for simultaneous inoculation.

The temperature at which seedlings were grown prior to inoculation had little effect on seedling reactions. Flecking was a little more distinct on resistant varieties grown at high temperatures prior to inoculation than on those grown on low temperatures.

Cause of the seasonal shift in reaction types.—Valentine and Rabat, which were included in all inoculation series, were the only varieties available for determining when the host response shifted from that typical of winter to that typical of spring. Valentine showed little response to season except for increased flecking in the spring. The beginning of the shift toward resistance, first noted on Rabat in late February and early March at a day length of about 10.6 hours, became distinct after late March at a day length of 11.6 hours.

The seedling reactions of Valentine and Rabat were studied under reduced day length and reduced light in an attempt to duplicate winter conditions. Seedings were grown at 18° C previous to inoculation under 2 day-length treatments: 1) an 8-hour day and 2) normal day length (approximately 11 hours). Inoculations were made with race 56 at 28° C on March 9. Following inoculation, 4 treatment combinations of day length and light intensity were used for each of the short-day and normal-day preinoculation groups. Each treatment combination was duplicated. The treatments were 1) normal day, normal light; 2) normal day, low light; 3) short day, normal light; and 4) short day, low light. Plants were covered with cages of 1 thickness of cheesecloth for the reduced-light treatment and covered with black paper cages from 4 p.m. to 8 a.m. to reduce the light period to 8 hours.

The moderately susceptible reaction of Rabat obtained earlier in the winter was not produced in any of the 8 treatments. Moderately small pustules, 2– to 2 type, occurred in green islands on Rabat in all

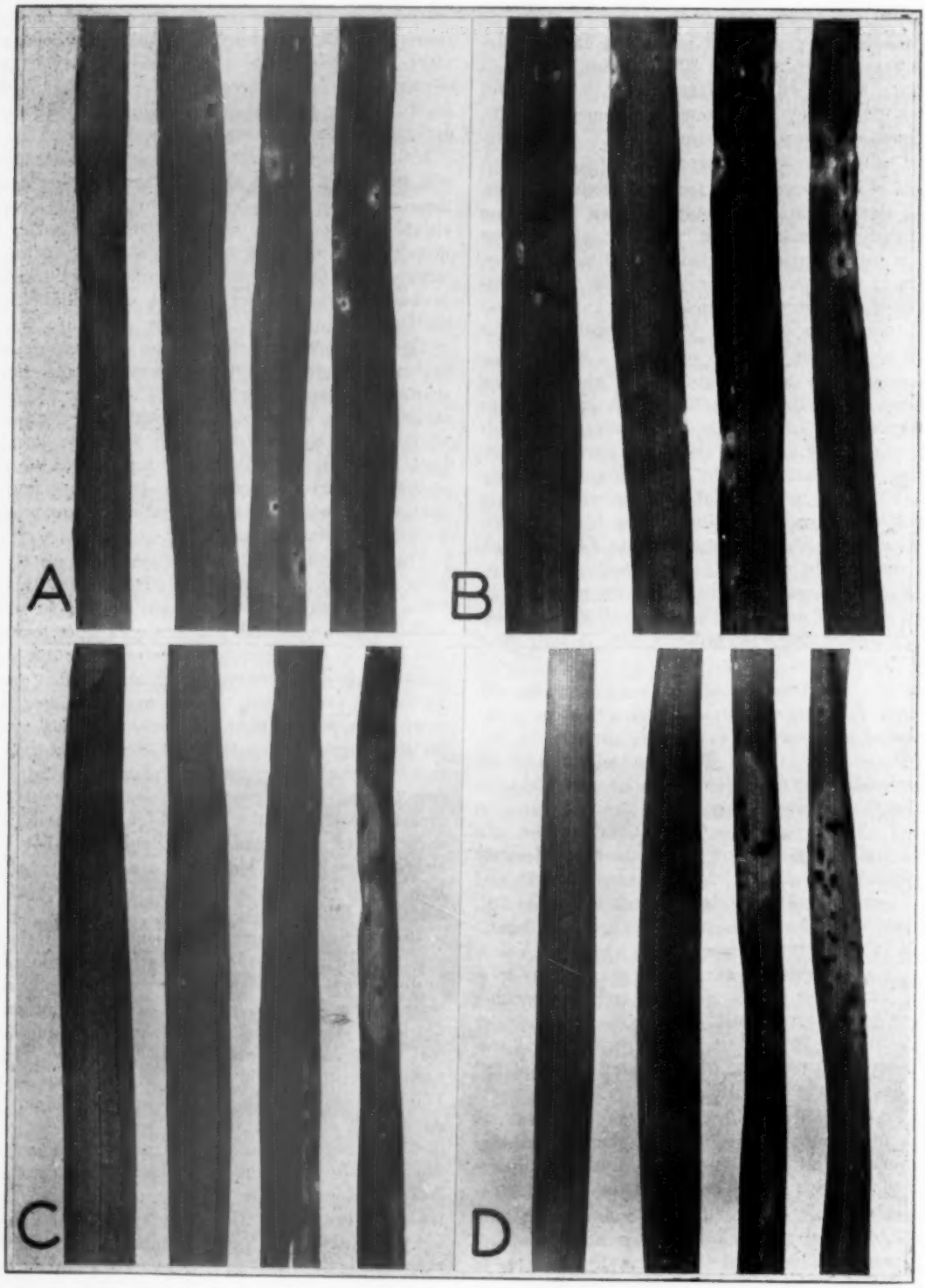


FIG. 1. Seedling reactions in winter of Valentine (left 2 leaves) and Rabat (right 2 leaves) to race 17 of *P. graminis* f. sp. *tritici* at A) 16°, B) 20°, C) 24° and D) 28°C.

treatments. Reduced-light or reduced-day-length treatments (or both) following inoculation resulted in pustules that were slightly smaller than when conditions were normal; thus, there was a greater frequency of 2⁻ type pustules. Shortened day length before inoculation had no observable effect on seedling reactions.

Valentine was highly resistant in all treatments.

Seedling vigor was noticeably reduced by either reduced-light or reduced-day-length treatments after inoculation and was greatly reduced by the combination of the 2 treatments.

Based on the results of this experiment, seasonal changes in rust reaction type on Rabat could not be attributed directly to changes in day length or in light intensity. Reduction of day length and light intensity resulted in a slight decrease in susceptibility of Rabat. Since pustule size is related to seedling vigor, additional treatment levels should be explored before ruling out day length and light intensity as the cause of seasonal changes in reaction types.

Differential response to races 17, 19, and 56 of P. graminis f. sp. *tritici*.—Differences in seedling response of barley varieties to races 17, 19, and 56 of *P. graminis* f. sp. *tritici* were obtained in the winter series. The differences in reaction were often slight and usually associated with the relative frequency of reactions common to all 3 races. In some cases, differential race response was evident only at certain temperatures. No evidence of high resistance to 1 race and high susceptibility to another was found. Responses to races 17 and 56 were similar, but they differed from the responses to race 19.

Typical reactions of 5 varieties among 52 varieties tested are presented in Table 2. A number of varieties, such as Valentine and Rabat, showed little differential response to races. Some, such as C.I. 6614, indicated a slight differential, with more or larger pustules appearing with race 19. Others, such as C.I. 7160, indicated larger differences, especially at certain temperatures. The C.I. 7163 variety showed a great difference in response to races at all temperatures, but

TABLE 1.—*Seasonal changes in seedling reactions of 10 barley varieties to race 17 of Puccinia graminis f. sp. tritici* [a]

	Rust reactions in tests made at indicated greenhouse temperatures during 2 seasons							
	16°C		20°C		24°C		28°C	
Variety	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring
Group 1, inoculated Dec. 17 and April 6								
Rabat	2⁻/5	0; 2⁻–2⁻/18	2⁻–2⁺⁺/20 3⁻/2	2⁻–2/47	2⁺/10 3⁻–/19	2⁻–2⁻/81	2⁺⁺/11 3–3⁺/20	2⁻–2⁻/57
Valentine	0–0;	0;	0–0;	0;	0–0;	0;	0	0;
Heitpas-5	0–0; 2⁻/2	0–0;	3⁻/3	0; 2⁻–2⁻/40	2/2 3/8	2⁻–2⁻/47	3⁻/2	2⁻/35
Chevron	0–0;	0;	0–0;	0;	0–0;	0;	0	0;
C.I. 6609	0–0; 2⁻/2	0; 2⁻/1	0–0; 2⁻/15	0;	0–0; 2⁻/1	0;	0 2⁻/10	0;
C.I. 6610	0–0; 2/3	0; 2⁻–2⁺/3	0; 1⁻/1 2⁻/11	0; 2⁻/26	1⁻/10 2⁻/10	0–0;	2⁻/25	0–0;
Group 2, inoculated March 3 and April 8								
Rabat	0; 1⁻/20 2⁻/120	0; 1⁻/7 2⁻–2⁻/28	1/4 2⁻–2/111	2⁻–2/53	2/79	2⁻–2/105	2⁺/23 3⁻–3/169	2⁻–2/71
Valentine	0; 1⁻/9 2⁻/8	0;	0;	0;	0;	0;	0;	0;
Kindred	0; 2⁻/4	0;	0;	0;	0;	0; 2⁻/2	0; 2⁻/2	0;
Moore	2–2⁺⁺/88	2⁻/18	2⁻–2⁺/61	2⁻–2⁻/26	2⁻–2⁻/42	0;	0; 2⁻–2⁻/65	0;
Trebi	2⁻–2/88	2⁻–2/38	2⁻–2/131	2⁻–2⁺/65	2⁻–2/97	2⁻–2⁻/44	2⁺–2⁺⁺/90 3⁻/14	2⁻–2⁻/93
Oderbrucker	2⁻–2/94	2⁻–2/64	2–2⁺/93 3⁻/3	2⁻–2⁺⁺/72	2⁺/41 3⁻–3/50	2⁻–2⁻/93	3⁺⁺/110	2⁻–2⁺/52 3⁻/1

[a] Seedling reactions are expressed as the numerator and the average number of pustules of the reaction type per 10 leaves as the denominator. Where 2 or more distinct reaction types occur on the same variety, they are entered on separate lines in the table. The number of pustules on the susceptible variety Rabat indicates the approximate amount of infection with each race.

Infection types are classified: 0 = immune, 0; = immune with hypersensitive flecks, 1 = very resistant, 2 = moderately resistant, 3 = moderately susceptible, and 4 = very susceptible. Plus and minus signs are used as superscripts to indicate variation within a given type; ⁺⁺ and ⁻ indicate the upper and lower limits, respectively, of each type.

differences were most pronounced at 20° and 24° C.

Single-pustule isolations of race 17 and race 19 of *P. graminis* f. sp. *tritici* were made from large and small pustules on Rabat. Five cultures from each

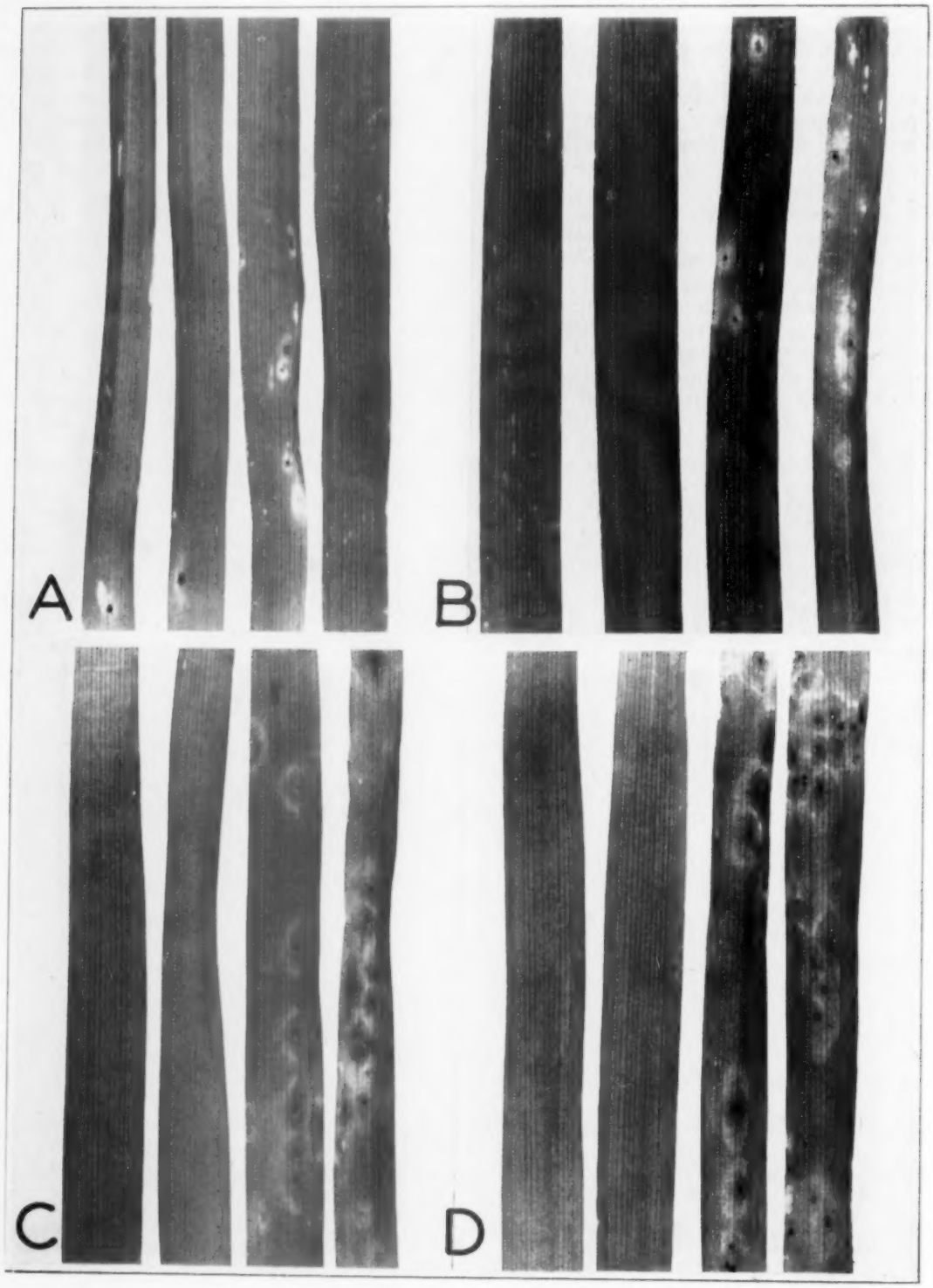


FIG. 2. Seedling reactions in spring of Valentine (left 2 leaves) and Rabat (right 2 leaves) to race 17 of *P. graminis* f. sp. *tritici* at A) 16°, B) 20°, C) 24° and D) 28°C.

TABLE 2.—*Seedling reactions of 5 barley varieties to races 17 and 19 of Puccinia graminis f. sp. tritici at 4 greenhouse temperatures in winter* [a]

Variety	Rust reactions in tests made at indicated greenhouse temperatures with 2 races							
	16°C		20°C		24°C		28°C	
	Race 17	Race 19	Race 17	Race 19	Race 17	Race 19	Race 17	Race 19
Rabat	2=–2⁻/31	2=–2⁻/33	2⁻–2/15	2–2⁺/12	2–2⁺⁺/13 3=–3/12	2–2⁺⁺/12 3⁻/18	2–2⁺⁺/14 3⁻–3/14	2–2⁺⁺/9 3/20
Valentine	0–0; 2⁻/8	0–0; 2⁻/10	0–0;	0–0;	0–0;	0–0;	0–0; 2=/2	0–0; 2=/2
C.I. 6614	0; 2⁻/3	0; 2⁻/3	0; 2⁻/3	0; 2=–2⁻/23	0; 2=/5	0; 2=/19	0;	0;
C.I. 7160	0; 2–2⁺⁺/7	0; 2⁻–2⁺/30	0; 2=–2/12	2–2⁺⁺/33	2=/17	2/31	0; 2=/2	2=–2⁺/32
C.I. 7163	0; 2⁺/3	2⁻–2/22	0;	2⁻–2/22	0;	2⁻/29	0; 2=/2	0; 2⁻/46

[a] See footnote Table 1.

race, the original cultures of races 17 and 19, and a race of *P. graminis* f. sp. *secalis* were compared at 28° C on the 4 varieties, Valentine, Rabat, Moore, and Heitpas-5. Little or no difference among isolates within races occurred. The range of reaction types on a variety was not reduced by further selection within the races. The unidentified race of *P. graminis* f. sp. *secalis* was somewhat more virulent on Valentine and Moore than were the 12 cultures of *P. graminis* f. sp. *tritici* tested. Reactions on Valentine were 0; for *P. graminis* f. sp. *tritici* and 0; and 2= for *P. graminis* f. sp. *secalis*. Reactions on Moore were 0; and 2= for *P. graminis* f. sp. *tritici* and 2= to 2 for *P. graminis* f. sp. *secalis*.

Comparison of seedling and adult-plant reactions.—Seedling and adult-plant reactions in the greenhouse were compared for Valentine, C.I. 6613, and C.I. 7163, all resistant in the field, and for Rabat, a variety susceptible in the field. Following the determination of seedling reactions at 16°, 20°, 24°, and 28° C, 32 plants of each variety grown at 16° were transplanted to 6-in. pots and held at 16° until about 2 weeks before heading. Eight plants of each variety were then placed in each of the temperature houses at 16°, 20°, 24°, and 28° for 16–20 days, when they had reached the early heading stage. The plants were then inoculated by spraying a suspension of urediospores of race 56 of *P. graminis* f. sp. *tritici* on the leaf sheaths from which the bloom had previously been removed. The inoculated plants were placed in moist chambers for 48 hours in the temperature house in which they were growing and then held at the same temperature until pustules were fully developed.

Valentine, C.I. 6613, and C.I. 7163, interpreted as possessing different degrees of resistance in the seedling stage (Table 2), were highly resistant in the adult-plant stage at each temperature. Rabat was susceptible in the adult-plant stage at all temperatures.

Seedling reactions also were compared with adult-plant reactions in the field. All but one of the 46 varieties selected for the experiment for adult-plant resistance were resistant in single-race and composite-race isolation nurseries of races 17, 19, and 56 in the field in each of 2 years. These 45 may be placed into 4 groups (Tables 1, 2) for seedling reaction: 1) varieties equal to Valentine in resistance; 2) varieties nearly equal to Valentine but having more pustules along with flecking (represented by C.I. 6609 in Table 1); 3) varieties having considerably more pustules than Valentine in winter tests and slight variation in reaction to races (represented by C.I. 6614 in Table 2); and 4) varieties with distinct differential response to races and no 0; reactions at certain temperatures (represented by C.I. 7160 in Table 2).

Heitpas-5 (C.I. 7124) was susceptible in the seedling stage at all temperatures but was resistant, moderately susceptible, or susceptible in the field depending upon environmental conditions. Shands and Wiebe (unpublished data obtained at Madison, Wisconsin) observed that the major-factor pair governing the stem rust reaction of Heitpas-5 was different than the major-factor pair *Tt* governing stem rust reaction in Chevron (and Valentine).

Other varieties susceptible in the adult-plant stage in the field were susceptible in the seedling stage and responded to temperature and season in a way similar to Rabat (Trebi and Oderbrucker in Table 1).

F_2 seedling reactions of Rabat × Valentine were compared with adult-plant reactions in the field. A total of 879 seedlings, 215–225 at each of 4 temperatures (16°, 20°, 24°, and 28° C), were classified following inoculation in February with race 56. Segregation at 24° and 28° was distinct and approximated 3 highly resistant to 1 susceptible. The plants also were tested for adult-plant resistance in the field. All plants highly resistant in the seedling stage were highly resistant in the adult-plant stage, and all plants susceptible in the seedling stage were susceptible in the adult-plant stage. At 16° and 20°, the F_2 segregation was not distinct, and some pustules developed on the Valentine parent. F_2 seedlings low in pustule number could not be placed with confidence into either resistant or susceptible classes.

DISCUSSION.—The most favorable temperature for

infection of barley with races 17, 19, or 56 of *P. graminis* f. sp. *tritici* was higher than that found by Johnson and Newton (4) for infection of Little Club wheat with any of several races of *P. graminis* f. sp. *tritici*, including races 19 and 56. Using temperatures ranging from 55° to 99° F (approximately 13° to 37° C), they noted that the higher the temperature at which plants were kept, the lower the pustule number.

The relation of temperature to incubation period of *P. graminis* f. sp. *tritici* for barley differed from that found by Stakman and Levine (6) for wheat. They found a lengthening of incubation period of about 1 day for every 10° F above the optimum temperature of 66.5°–70° F.

These responses appear to be differences between crop species but since different isolates of races were used for the 2 crops, the possibility of differences between isolates of the same race was not eliminated.

The variability of reaction types on single leaves obtained on barley seedlings in the greenhouse suggests several problems for further investigation. This variability may be attributable to varietal characteristics, but general chlorosis of susceptible seedlings and lack of complete susceptibility in the seedling reaction suggest that testing conditions may not have been optimum. Seedling reactions varied less, and differences between varieties resistant and those susceptible in the adult-plant stage in the field were usually more distinct, at constant air temperatures of 28° or 24° C than at 20° or 16°. Modification of nutrition, light intensity, and day length at these higher temperatures might improve testing conditions. Cool night temperatures also have received inadequate attention.

Seedling reactions of barley to stem rust were greatly influenced by temperature and season. A division between seedling reactions corresponding to susceptible adult-plant reactions and those corresponding to resistant adult-plant reactions must take into consideration season and temperature and even varieties to some extent. Valentine and Rabat and their F_2 hybrid populations could be classified accurately for resistance or susceptibility by seedling reactions at 24° or 28° C in either winter or spring tests. The difference between Spartan, a variety susceptible in the adult-plant stage, and C.I. 7174, a variety resistant in the adult-plant stage, was not easily detected in the seedling stage in winter but was clearly evident at 24° and 28° C in the spring and fall. The type of adult-plant resistance possessed by Heitpas-5 could not be detected in the seedling stage. A study of parental and hybrid seedling reactions at different temperatures and in different seasons should be helpful in determining the conditions under which the most effective selection can be made in breeding for resistance.

WISCONSIN AGRICULTURAL EXPERIMENT STATION
MADISON, WISCONSIN

LITERATURE CITED

1. ALI, S. B. 1954. Behavior of isolates of race 59 of Puccinia graminis tritici on certain varieties of wheat and barley. Phytopathology (Abs.) **44**: 481.
2. ANDREWS, J. E. 1954. Inheritance of reactions to loose smut and to stem rust in barley. (Abs.) Dissertation Abstr. **14**: 899.
3. JOHNSON, T., AND K. W. BUCHANNON. 1954. The reaction of barley varieties to rye stem rust, Puccinia graminis var. secalis. Can. J. Agr. Sci. **34**: 473-482.
4. JOHNSON, T., AND MARGARET NEWTON. 1937. The effect of high temperature on uredial development in cereal rusts. Can. J. Research, Sect. C. **15**: 425-432.
5. MILLER, J. D., AND J. W. LAMBERT. 1955. Variability and inheritance of reaction of barley to race 15B of stem rust. Agron. J. **47**: 373-377.
6. STAKMAN, E. C., AND M. N. LEVINE. 1919. Effect of certain ecological factors on the morphology of the urediniospores of Puccinia graminis. J. Agr. Research **16**: 43-77.
7. STAKMAN, E. C., M. N. LEVINE, AND W. Q. LOEGERING. 1944. Identification of physiologic races of Puccinia graminis tritici. U. S. Dep. Agr. Bur. Entomol. Plant Quarantine, E. T. No. E-617. 27 p.
8. WATERHOUSE, W. L. 1947. Studies in the inheritance of resistance to rust of barley. Part II. J. Proc. Roy. Soc. N. S. Wales **81**: 198-205.

VENTURIA INAEQUALIS (CKE.) WINT. XII. GENES CONTROLLING PATHOGENICITY OF WILD-TYPE LINES [1]

D. M. Boone and G. W. Keitt

SUMMARY

Ten monosporic lines of *Venturia inaequalis* (Cke.) Wint. from 7 different states were tested for pathogenicity to the leaves of 15 apple varieties. The lines differed in their pathogenicity to the several varieties, inciting typical lesions on some and only flecks on others; however, certain lines were similar in pathogenicity to certain varieties or groups of varieties. Studies of the inheritance of pathogenicity showed that each of 7 or more gene pairs of the fungus controlled pathogenicity to 1 or more of the apple varieties studied. Each pair consisted of an allele conditioning the lesion reaction and another conditioning the fleck reaction. Four of these gene pairs were found to be at separate loci. The remaining pairs also are tentatively considered to be at separate loci, but genetic proof is yet incomplete. The gene pairs and the varieties to which they control pathogenicity are as follows: 1) McIntosh, Yellow Transparent, and Hibernal; 2) Haralson, Wealthy, Prairie Spy, Beacon, Rome Beauty, and Northwestern Greening; 3) Red Astrachan and Yellow Transparent; 4) Haralson, Hyslop, and Yellow Transparent; 5) Hyslop; 6) Grimes Golden; and 7) Prairie Spy. Gene pair 4 was found to be approximately 13 crossover units from its centromere; all the other pairs appear to be 33 units or more from their respective centromeres. In those cases in which gene pairs at different loci controlled pathogenicity to the same variety, the alleles conditioning flecks were epistatic to those conditioning lesions.

This paper is one of a series dealing with the genetics, nutrition, and pathogenicity of the apple scab fungus, *Venturia inaequalis* (Cke.) Wint. The present study is concerned primarily with inheritance of pathogenicity. The background of the work and pertinent literature have been reviewed by Keitt (6, 7).

Keitt and Palmiter (15) and Keitt and Langford (11) found that factors controlling pathogenicity usually segregated in 1:1 ratio in the asci. Keitt et al (12) reported finding only 2 major types of pathogenic reaction, namely "lesion" (typically pathogenic with abundant sporulation of the fungus) and "fleck" (nonpathogenic or only slightly pathogenic, with sporulation lacking or very sparse). In all adequately studied cases, pathogenicity, as indicated by the lesion and the fleck reactions, was inherited as if single-gene controlled. Keitt et al (14) showed that pathogenicity to the apple varieties McIntosh and Yellow Transparent was controlled by 1 gene pair and pathogenicity to Haralson and Wealthy by a second gene pair, which assorted independently of the first.

The present study is an extension of this earlier work and involves different wild-type lines and additional apple varieties. The primary purposes were to search for additional genes controlling pathogenicity, to determine whether there are few or many such genes, and to locate them with reference to their respective centromeres or to other marker genes. Evidence is given for 5 more gene pairs, each controlling pathogenicity to a certain apple variety or a group of varieties. The inheritance and some effects of all 7 gene pairs are reported. Preliminary accounts of a part of this work have been given (6, 7, 8, 9, 10).

MATERIALS AND METHODS.—*Lines employed.*—The sources of the parental lines are given in Table 1. Lines D5 (11), 365-4 (2), 360-4, and 422-3 originated as monoascosporic isolates from perithecia in overwintered apple leaves. Line 1031-4 (2) is from a monoascosporic isolate out of a perithecium produced *in vitro*. The remaining lines are from monoconidial isolates from living leaves.

Cultural and inoculation methods.—The lines were maintained routinely by monoconidial transfers made at 8-week intervals to tube slants of modified potato-

TABLE 1.—*Sources of the parental lines of Venturia inaequalis*

Line	Year isolated	Variety from which isolated	State from which collected
D5	1937	McIntosh	Wisconsin
360-4	1942	Siberian crab	Oregon
365-4	1942	Dudley	Wisconsin
422-3	1942	Rome Beauty	Missouri
1031-4 [a]	1946		
1070	1946	Winesap	Tennessee
1071	1946	?	Arkansas
1074	1946	Rhode Island Greening	New York
1096	1946	*Malus arnoldiana*	Illinois
1098	1946	*Malus floribunda*	Illinois

[a] From a perithecium produced *in vitro* by a cross of 2 lines isolated from the Dudley variety in Wisconsin.

[1] Accepted for publication January 28, 1957.
Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
This work was supported in part by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation, by Grant E-140 (C) from the National Microbiological Institute of the National Institutes of Health, Public Health Service, and by Grant G-661 from the National Science Foundation.
Grateful acknowledgements are made to C. Leben for counsel in the earlier phase of the study, to J. C. Horton, N. W. Marty, H. A. Lamey, and D. M. Kline for assistance in the laboratory and greenhouse work, and to E. H. Herrling for preparing the illustration.

TABLE 2.—*Pathogenic reactions incited with the different apple varieties by the lines of Venturia inaequalis*

Variety	Lines and pathogenic reaction[a]									
	365-4	1098	1071	422-3	D5	1074	360-4	1031-4	1096	1070
McIntosh	L	L	L	L	L	L	F	F	F	F
Yellow Transparent	L	L	L	F	F	F	F	F	F	F
Hibernal	L	L	L	F	F	F	F	F	F	F
Red Astrachan	L			F	F	F		L	L	
Haralson	L	L	L	L	L	L	F	F	F	F
Wealthy	L	L	L	L	L	L	F	F	F	F
Prairie Spy	L	L	L	L	L	L	F	F	F	F
Beacon	L	L	L	L	F	L	F	F	F	L
Northwestern Greening	L	L	L	L	L	L	F	F	F	L
Hyslop	L	L	F	L	L	F	L	L	L	F
Grimes Golden	L	L	F	F	L	L	F	L	L	L
Cortland	L	L	L	L	L	L	L	L	L	L
Red Delicious	L	L	L	L	L	L	L	L	L	L
Duchesss	L	F	F	0	F	0	0	F	F	0
Whitney	F	F	F	F	—	F	F	F	F	F

[a] L = lesion; F = fleck; 0 = no macroscopic symptoms; — = no data.

dextrose agar (2); these slants were incubated at 16°C. Reserve stock cultures were stored under mineral oil. Matings were cultured and ascospores isolated by the method of Boone and Keitt (1) as modified from the methods of Keitt and Langford (11). Potato-dextrose medium was substituted for malt-extract medium, but otherwise the methods of Keitt and Langford (11) and Keitt et al (12) were employed in growing and preparing inocula, in potting, forcing, inoculating, and incubating the trees, and in taking records on pathogenic reactions. The pathogenicity tests were repeated if necessary in order to obtain definitive symptom expression. Ordinarily, in tests with the progenies of crosses, all 8 cultures from an ascus were used so that the tests of the chromatid mates served as replications.

RESULTS.—*Pathogenicity of the parental lines.*—The pathogenic reactions incited with the different apple varieties by the parental lines are indicated in Table 2. The reactions were classified for the purposes of this study as being either the lesion or the fleck type although there was variation in lesion size, in the abundance of sporulation in the lesions, and in the size and demarcation of the flecks. These reactions varied according to the particular isolate-variety combinations employed.

There were important differences in the pathogenic capabilities of the lines. Lines such as 365-4 and 1098 incited lesions on most of the varieties used, whereas others such as 360-4, 1031-4, 1070, and 1096 incited only flecks on many of the varieties; however, all lines were alike in being highly pathogenic to Cortland and Red Delicious and nonpathogenic or only slightly pathogenic to Duchess and Whitney.

Certain of the lines showed similarity in their inability to incite lesions on certain groups of varieties, e.g. lines 360-4, 1031-4, 1070, and 1096 incited only flecks on the varieties McIntosh, Yellow Transparent, and Hibernal. Similarly, lines D5, 422-3, and 1074 incited flecks on Red Astrachan, Yellow Transparent, and Hibernal, and lines 360-4, 1031-4, and 1096 incited flecks on Haralson, Wealthy, Beacon, Northwestern Greening, and Prairie Spy. These results suggested that pathogenicity to all members of each group of varieties was controlled in each line by the same gene and that the lines showing similar pathogenicity carried certain identical pathogenicity genes. This was further indicated by the results from the study of the pathogenicity of the progenies from crosses between these lines.

Inheritance of pathogenicity.—With a few exceptions noted later, pathogenicity to a given variety was inherited in each cross as if controlled by a single gene. To test for single-gene control of pathogenicity to a variety, lines inciting flecks on it were crossed with lines inciting lesions. Single-gene control was indicated if 4 of the cultures from each resulting ascus incited the fleck reaction and the other 4 the lesion reaction (see segregation for pathogenicity to Haralson, McIntosh, and Red Astrachan in Table 3).

Certain individual gene pairs (or possibly, very closely linked gene pairs) were found to control pathogenicity to more than 1 variety. To determine

TABLE 3.—*Pathogenic reactions incited by the monoascosporic isolates of 3 asci from the cross D5 × 1031-4*[a]

Ascus	Apple variety	Ascosporic isolate and pathogenic reaction[b]							
		1	2	3	4	5	6	7	8
1305	Haralson	L	L	L	L	F	F	F	F
	McIntosh	L	L	L	L	F	F	F	F
	Yellow Transparent	F	F	F	F	F	F	F	F
	Red Astrachan	F	F	F	F	L	L	L	L
1306	Haralson	L	L	L	L	F	F	F	F
	McIntosh	F	F	L	L	F	F	L	L
	Yellow Transparent	F	F	L	L	F	F	L	L
	Red Astrachan	F	F	L	L	F	F	L	L
1326	Haralson	L	L	F	F	F	F	L	L
	McIntosh	F	F	L	L	L	L	F	F
	Yellow Transparent	F	F	F	F	L	L	F	F
	Red Astrachan	L	L	F	F	L	L	F	F

[a] Line D5 incited flecks on Red Astrachan and Yellow Transparent and lesions on Haralson and McIntosh; line 1031-4 incited flecks on Haralson, McIntosh, and Yellow Transparent and lesions on Red Astrachan.

[b] L = lesion; F = fleck.

this, the progeny from a cross was tested on all the varieties on which 1 parent incited flecks and the other parent, lesions. Control of pathogenicity to all the varieties by the same gene pair was indicated if each progeny culture incited either the lesion reaction on all the varieties or the fleck reaction on all. If some of the progeny cultures incited lesions on some of the varieties and flecks on the others, however, it was evidence that genes at 2 or more loci were involved; e.g. although line 1031-4 incited flecks on 8 varieties, study of the progeny of this line crossed with line 365-4 revealed that pathogenicity to 3 of the varieties was controlled at 1 locus and to the other 5 at another.

Several examples were found of genes at different loci conditioning the fleck reaction with the same variety. To determine this, the progeny from the cross between lines inciting the fleck reaction on a variety was tested on the variety. If some of the progeny cultures incited lesions, it was evidence that in 1 parent the gene conditioning the fleck reaction was at a different locus than was the fleck-conditioning gene in the other parent (see segregation for pathogenicity to Yellow Transparent in Table 3). If all the progeny cultures incited flecks, however, it was evidence that the genes were at the same locus.

Three lines of evidence indicated that in the different lines certain of the genes conditioning flecks were at the same locus: 1) the genes controlled pathogenicity to the same variety or group of varieties, 2) they segregated in the second nuclear division in the ascus with approximately the same frequency, and 3) they did not recombine when in the same cross. Tables 4 and 5 are summaries of the data on the segregation and recombination of the genes. In a few cases, data on recombination were not available for genes thought from the other 2 lines of evidence to be at the same locus.

Pathogenicity genes.—The results of the pathogenicity tests with the parental lines and their progenies indicated that 7 or more different gene pairs in the fungus lines conditioned the lesion and fleck reactions with the apple varieties employed. Each pair consisted of an allele conditioning the lesion reaction and another the fleck reaction with 1 or more varieties. Some of these gene pairs have been referred to formerly (10) by the names of differential varieties used to identify them. In the present paper each is referred to by a number. These gene pairs are tentatively considered to be at separate loci although genetic proof of this is as yet incomplete. The genes are called "pathogenicity genes" and have been assigned the genetic symbol "*p*."[2] Genes at different loci are

[2] Genetic symbols were chosen in joint conferences with J. R. Shay and E. B. Williams of Purdue University and with P. R. Day of the John Innes Horticultural Institution, England.

TABLE 4.—*Summary of the data on segregation of the pathogenicity gene pairs*

Gene pair	Source of fleck allele	Number of asci studied	Percentage of second-division segregation[a]
1	365-1[b]	35[b]	77.1
1	360-4	13	76.9
1	1031-4	69	75.3
1	1070	21	81.0
1	1096	9	88.9
Total or mean		147	77.6[c]**
2	454-2,6[b]	35[b]	77.1
2	360-4	9	88.9
2	1031-4	77	80.5**
2	1096	11	100.0*
Total or mean		132	81.8[c]**
3	D5	54	74.1
3	422-3	2	100.0
3	1074	6	66.7
Total or mean		62	74.2[c]
4	1070	46	26.1**
5	1074	9	88.9
6	422-3	13	76.9
7	1070	14	85.7

[a] Tested by Chi-square for deviation from 66.7 per cent (2 second-division segregations: 1 first-division segregation). Double asterisks indicate significant deviation at 1 per cent level, single at 5 per cent level.

[b] Keitt et al (14).

[c] Calculated for the total number of asci. Chi-square test indicated that the pooled data were homogeneous.

TABLE 5.—*Summary of the data on recombination of the pathogenicity genes*

Loci of genes		Sources of fleck-alleles		Observed tetrad numbers[a] PD	NPD	T	Percentage recombination[b]
1	1	365-1[c]	360-4	8	0	0	0.0**
1	1	365-1	1031-4	7	0	0	0.0**
1	1	1070	1096	2	0	0	0.0
1	2	365-1	454-2,6[c]	4	11	20	60.0
1	2	360-4	360-4	4	0	4	25.0
1	2	1031-4	1031-4	22	13	51	44.8
1	2	1096	1096	2	2	5	50.0
Mean for 1, 2 loci							47.8[d]
1	3	1031-4	D5	3	4	14	52.4
1	3	1070	1074	1	1	5	50.0
1	3	1096	D5	1	0	3	37.5
1	3	360-4	D5	1	0	1	25.0
Mean for 1, 3 loci							48.5[d]
1	4	1070	1070	2	1	17	47.5
2	2	454-6	360-4	9	0	0	0.0**
2	2	454-6	1031-4	8	0	0	0.0**
2	3	1031-4	D5	3	9	11	63.0
2	3	1096	D5	0	0	4	50.0
Mean for 2, 3 loci							61.1[d]
2	4	1096	1070	2	1	9	45.8
2	7	1096	1070	0	2	3	70.0
3	3	D5	422-3	3	0	0	0.0*
3	3	D5	1074	4	0	0	0.0*
3	4	1074	1070	0	2	4	66.7

[a] PD = parental ditype; NPD = nonparental ditype; T = tetratype.

[b] Based on half-tetrad numbers. Tested by Chi-square for deviation from 50.0 per cent (1 recombinant half-tetrad: 1 parental half-tetrad). Double asterisks indicate significant deviation at 1 per cent level, single at 5 per cent level.

[c] Keitt et al (14).

[d] Calculated for the total number of asci. Chi-square test indicated that the pooled data were homogeneous.

distinguished by numbers following the symbol and separated from it by a dash. The numbers used are the same as those of the 7 gene pairs. Lesion-conditioning alleles are distinguished from fleck-conditioning alleles by the superscript "+." Thus a line with the genotype *p*–1$^+$ *p*–2 *p*–3 carries the lesion-conditioning allele at pathogenicity locus 1 and the fleck-conditioning alleles at loci 2 and 3.

The gene pairs and the varieties to which they control pathogenicity are as follows:

1) First described by Keitt et al (14) as controlling pathogenicity to McIntosh (Fig. 1, 2) and Yellow Transparent (Fig. 7). The present study showed that pathogenicity to Hibernal is also controlled by genes of this pair. Lines 360-4, 1031-4, 1070, and 1096 appear to have identical *p*–1 genes. Those of 360-4 and 1031-4 were tested for recombination with the fleck-conditioning allele of line 365-1 of Keitt et al (14), and none was found.

2) First described by Keitt et al (14) as controlling pathogenicity to Haralson (Fig. 3) and Wealthy. The present study revealed that Beacon, Prairie Spy (Fig. 8), Northwestern Greening, and Rome Beauty also belong in this group of varieties. Lines 360-4, 1031-4, and 1096 appear to have identical *p*–2 genes. Those of 360-4 and 1031-4 were tested for recombination with the fleck-conditioning allele of lines 454-2 and 454-6 of Keitt et al (14), and none was found.

3) Controls pathogenicity to Red Astrachan (Fig. 4) and Yellow Transparent. The results from the study of the parental lines indicated that pathogenicity to Hibernal may also be controlled by this gene pair, but this possibility was not explored further in the studies with the progenies. Flecks on Yellow Transparent conditioned by *p*–3 could not be distinguished from those conditioned by *p*–1. Lines D5, 422-3, and 1074 appear to have identical *p*–3 genes.

4) Controls pathogenicity to Haralson and Hyslop. There was limited evidence that pathogenicity to Yellow Transparent also is controlled by this pair. Flecks conditioned with Haralson by *p*–4 were not as chlorotic nor as sharply delimited as those conditioned by *p*–2.

5) Controls pathogenicity to Hyslop (5). Flecks conditioned by *p*–5 were sometimes large and dendritic in outline but always sharply delimited. On older, more resistant leaves they were usually only small pit-like depressions in the leaf surface.

6) Controls pathogenicity to Grimes Golden (Fig. 6). Flecks conditioned by *p*–6 varied somewhat in size according to the age of the leaves infected, the flecks on the younger leaves often being large with sparse sporulation in their centers.

7) Controls pathogenicity to Prairie Spy (Fig. 9). Flecks conditioned by *p*–7 were larger and more diffuse than those conditioned by *p*–2 with this variety (see Fig. 8, 9).

No conclusions were reached as to the identity or to the number of genes in lines 1071 and 360-4 controlling pathogenicity to Grimes Golden. Both lines incited distinctive flecks; however, the pathogenic reactions incited by some of the progeny cultures from a cross of line 1071 with 1074 could not be classified readily as being of the fleck type nor of the lesion type; the reactions on the older leaves were fleck-like but those on the younger leaves were large with considerable sporulation in them. The inheritance of the factor or factors in line 360-4 controlling pathogenicity to Grimes Golden was not studied.

Studies with lines 1070 and 1071 were not extensive enough to reveal the number of their genes conditioning flecking on Hyslop. There was evidence that in line 1070 there was another fleck-conditioning gene besides *p*–4. Study of the progeny of this line crossed with line 1074 revealed that the second gene was not *p*–5. There was also evidence of more than 1 fleck-conditioning gene in line 1071. Conclusions as to the total number of gene pairs in the lines controlling pathogenicity to Hyslop must await further study.

Pathogenicity loci.—The results (Tables 4, 5) of the studies of segregation and recombination of the 7 gene pairs indicated that pairs 1, 2, 3, and 4 are at separate loci. The other 3 pairs are tentatively considered to be also at separate loci, but genetic proof of this is incomplete.

Only pathogenicity locus 4 was located, by Lindegren's method (16), with reference to its centromere, approximately 13 crossover units from it. The percentages of second-division segregation for the other loci (Table 4) equalled or exceeded 66.7, indicating that they are approximately 33 units or more from their respective centromeres.

There were significant excesses of second-division segregations for gene pairs 1 and 2 (Table 4). Perkins (17) has discussed such excesses in relation to chiasma interference.

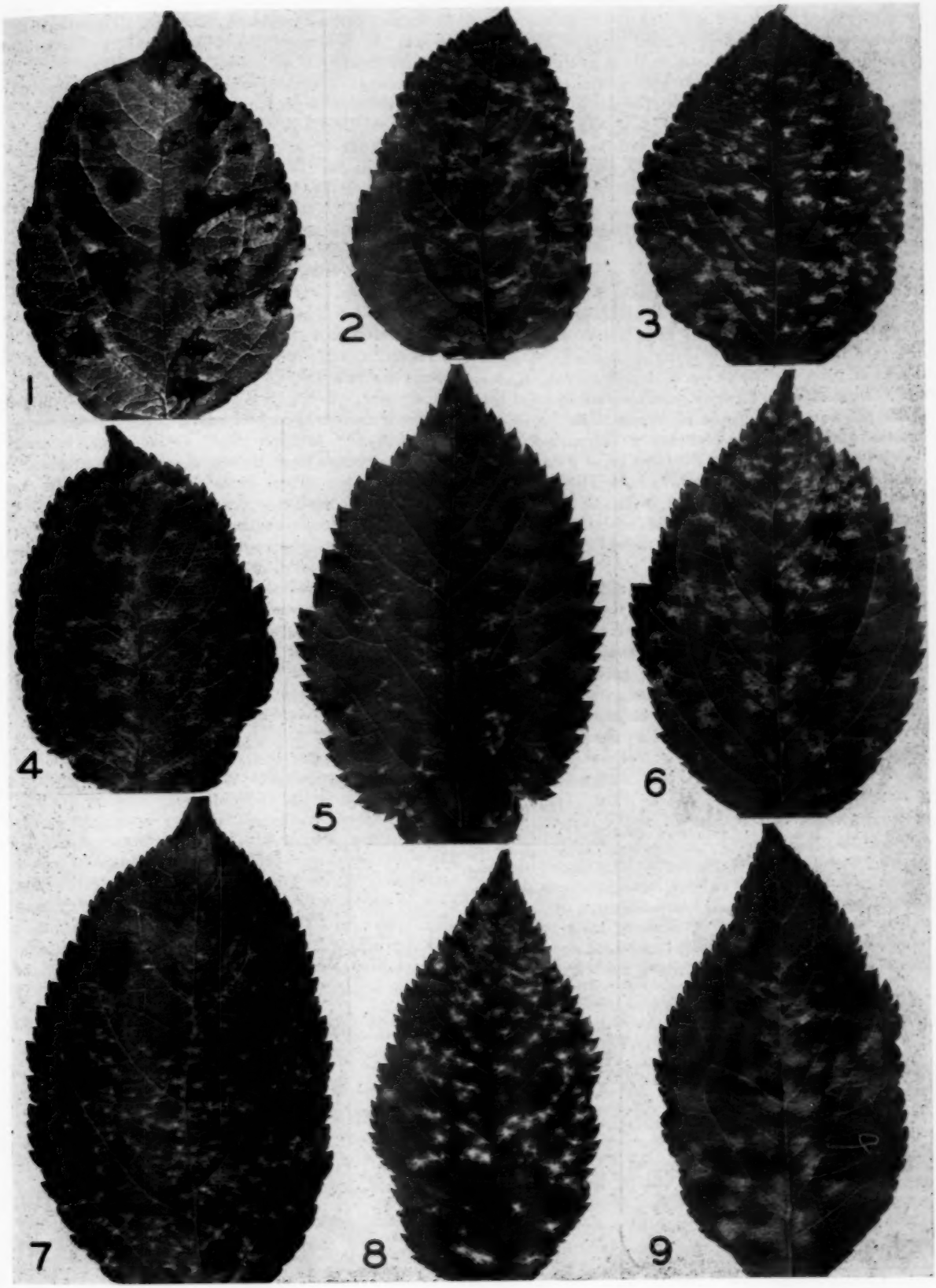
The available evidence (Table 5) indicates that loci 1, 2, 3, and 4 are not linked. Information concerning the linkage relationships among these 4 loci and the other 3 must await further tests of recombination.

In this study, additional evidence confirmed the finding of Keitt et al (13) that locus 1 is linked with the mating-type locus. Based on recombination data from 95 asci (37 PD; 2 NPD; 56 T), these 2 loci are 31.6 uncorrected map units apart.

Interaction of pathogenicity genes.—As shown in Table 3, ascosporic isolates carrying either the *p*–1

→

FIG. 1–9. Apple leaves inoculated with *Venturia inaequalis*.—Fig. 1. Lesions on McIntosh.—Fig. 2–9. Flecks on 7 varieties.—Fig. 2. McIntosh.—Fig. 3. Haralson.—Fig. 4. Red Astrachan.—Fig. 5. Hyslop.—Fig. 6. Grimes Golden.—Fig. 7. Yellow Transparent.—Fig. 8, 9. Prairie Spy.


1
2
3
4
5
6
7
8
9

or *p*–3 allele incited flecks on Yellow Transparent even though they carried p–3$^+$ or p–1$^+$ at the other pathogenicity locus. This indicated that the fleck-conditioning alleles were epistatic to those controlling the lesion reaction. Similarly, for pathogenicity to Haralson in other progenies, *p*–2 was epistatic to *p*–4$^+$, and *p*–4 to *p*–2$^+$; with Prairie Spy, *p*–2 was epistatic to *p*–7$^+$, and *p*–7 to *p*–2$^+$. These results and the lack of any asci with the 3:1 lesion/fleck segregation ratio in any of the crosses studied suggest that fleck-conditioning genes may be generally epistatic to all lesion-conditioning genes controlling pathogenicity to the same variety.

Differential apple varieties.—As a result of this study, 7 apple varieties were selected for further use in identifying the genotypes of wild-type lines with respect to the 7 pathogenicity gene pairs. These varieties are listed in Table 6 along with designations of the gene pairs for which each is differential. Under some circumstances, however, complete analysis of the genotypes cannot be made by using these varieties; i.e. the allele at locus 7 cannot be identified when *p*–2 is in the genotype, the allele at locus 5 cannot be identified when *p*–4 is present, and the allele at locus 4 cannot be identified when *p*–1, *p*–2, and *p*–5 are all present, etc. In such cases, the inheritance of the pathogenicity must be studied in order to identify the genotype.

Discussion.—The genes dealt with here are those that in wild-type lines determine whether the lesion or the fleck reaction will be incited with the leaves of a given apple variety or group of varieties. Although they are spoken of as controlling pathogenicity, it is recognized that the genotypes of both the pathogen and the host determine the expression of pathogenicity of the parasite within the limits imposed by the environment. The importance of the genotype of the pathogen is shown by the different reactions of fungus lines with 1 variety; the importance of the host genotype is shown by different reactions of apple varieties with 1 line of the pathogen.

The genes controlling pathogenicity can be identified only if different alleles at a locus condition different pathogenic reactions. Thus far, genes controlling pathogenicity to Cortland or Whitney have not been found because all lines tested on Cortland incited lesions and all tested on Whitney incited flecks. If more lines of the fungus were tested, one inciting flecks on Cortland might be discovered. Lines inciting lesions on Whitney should be found by isolating cultures from scab lesions on this variety.

By use of a limited number of fungus lines and apple varieties, 7 gene pairs controlling pathogenicity have been identified. It seems probable that use of a wider range of lines and varieties could reveal many more. Williams (20) has found 5 gene pairs controlling pathogenicity to different species of apple.

Widespread geographical distribution of certain pathogenicity genes was indicated. Apparently identical *p*–1 genes were found in lines from such widely separated states as Oregon, Wisconsin, and Tennessee, *p*–2 genes in lines from Oregon, Wisconsin, and Illinois, and *p*–3 genes in lines from Wisconsin, Missouri, and New York.

There is little point to classifying lines of *V. inaequalis* into races based on their pathogenic reactions with differential varieties of commercial apples (*Malus sylvestris*), none of which is known to possess field immunity to apple scab. *V. inaequalis* in most climates depends upon its sexual stage for its primary inoculum. This affords great opportunity for the development of new biotypes and sharply limits the possibility of developing races that will gain ascendancy and persist. Useful distinction of races has, however, been made by Shay and Williams (19) on the basis of differential pathogenicity to *Malus* species, some of which possess field immunity.

Attention is being given to the inheritance of resistance in the host. Dayton et al (3), Hough et al (5) and Shay et al (18) have shown that in certain scab-resistant selections or species of apple, there are examples of single-gene resistance and also multiple-gene resistance. They found resistance genes generally dominant to susceptibility genes. There is the interesting possibility that for each lesion- or fleck-conditioning gene in the pathogen there is a corresponding gene for susceptibility or resistance in the host. Such a relationship has been reported for the flax rust fungus and for flax by Flor (4). If such a condition exists in apple, it would mean that Yellow Transparent carries at least 3 resistance genes; Haralson, Hyslop, and Prairie Spy, 2 each; and McIntosh, Red Astrachan, and Grimes Golden, 1 each. Studies of the inheritance of resistance to apple scab in these varieties are in progress.

Table 6.—*Differential apple varieties used to identify pathogenicity genotypes of wild-type lines of Venturia inaequalis*

Variety	Gene pairs for which variety is differential						
	1	2	3	4	5	6	7
McIntosh	x						
Yellow Transparent	x		x	x			
Haralson		x		x			
Red Astrachan			x				
Hyslop				x	x		
Grimes Golden						x	
Prairie Spy		x					x

Department of Plant Pathology
University of Wisconsin
Madison, Wisconsin

LITERATURE CITED

1. Boone, D. M., and G. W. Keitt. 1956. Venturia inaequalis (Cke.) Wint. VIII. Inheritance of color mutant characters. Am. J. Botany **43**: 226-233.
2. Boone, D. M., J. F. Stauffer, M. A. Stahmann, and G. W. Keitt. 1956. Venturia inaequalis (Cke.) Wint. VII. Induction of mutants for studies on genetics, nutrition, and pathogenicity. Am. J. Botany **43**: 199-204.
3. Dayton, D. F., J. R. Shay, and L. F. Hough. 1953. Apple scab resistance from R12740-7A, a Russian

apple. Proc. Am. Soc. Hort. Sci. **62**: 334-340.
4. Flor, H. H. 1955. Host-parasite interaction in flax rust—its genetics and other implications. Phytopathology **45**: 680-685.
5. Hough, L. F., J. R. Shay, and D. F. Dayton. 1953. Apple scab resistance from Malus floribunda Sieb. Proc. Am. Soc. Hort. Sci. **62**: 341-347.
6. Keitt, G. W. 1952. Inheritance of pathogenicity in Venturia inaequalis (Cke.) Wint. Am. Naturalist **86**: 373-390.
7. Keitt, G. W. 1952. Inheritance of pathogenicity and some other characters in Venturia inaequalis. Advancement of Sci. [London] **9**(33): 39-44.
8. Keitt, G. W. 1953. Inheritance of pathogenicity in Venturia inaequalis. Proc. Intern. Botan. Congr., 7th Congr., Stockholm, 1950. p. 704.
9. Keitt, G. W., and D. M. Boone. 1951. Gene pairs conditioning pathogenicity in Venturia inaequalis. (Abs.) Phytopathology **41**: 19-20.
10. Keitt, G. W., and D. M. Boone. 1952. Additional gene pairs conditioning pathogenicity in Venturia inaequalis. (Abs.) Phytopathology **42**: 480.
11. Keitt, G. W., and M. H. Langford. 1941. Venturia inaequalis (Cke.) Wint. I. A groundwork for genetic studies. Am. J. Botany **28**: 805-820.
12. Keitt, G. W., M. H. Langford, and J. R. Shay. 1943. Venturia inaequalis (Cke.) Wint. II. Genetic studies on pathogenicity and certain mutant characters. Am. J. Botany **30**: 491-500.
13. Keitt, G. W., C. C. Leben, and J. R. Shay. 1946. Inheritance of pathogenicity and sex reaction in Venturia inaequalis. (Abs.) Phytopathology **36**: 403.
14. Keitt, G. W., C. C. Leben, and J. R. Shay. 1948. Venturia inaequalis (Cke.) Wint. IV. Further studies on the inheritance of pathogenicity. Am. J. Botany **35**: 334-336.
15. Keitt, G. W., and D. H. Palmiter. 1938. Heterothallism and variability in Venturia inaequalis. Am. J. Botany **25**: 338-345.
16. Lindegren, C. C. 1933. The genetics of Neurospora—III. Pure bred stocks and crossing-over in N. crassa. Bull. Torrey Botan. Club **60**: 133-154.
17. Perkins, D. D. 1955. Tetrads and crossing over. J. Cellular Comp. Physiol. **45** (Suppl. 2): 119-149.
18. Shay, J. R., D. F. Dayton, and L. F. Hough. 1953. Apple scab resistance from a number of Malus species. Proc. Am. Soc. Hort. Sci. **62**: 348-356.
19. Shay, J. R., and E. B. Williams. 1956. Identification of three physiological races of Venturia inaequalis. Phytopathology **46**: 190-193.
20. Williams, E. B. 1954. The inheritance of pathogenicity and certain other characters in Venturia inaequalis (Cke.) Wint. Ph.D. Thesis, Purdue University. 82 p.

LAMONT, A NEW VARIETY OF RESCUEGRASS RESISTANT TO HEAD SMUT[1]

Howard W. Johnson[2]

SUMMARY

Lamont rescuegrass is a selection made at Stoneville, Mississippi, from the "La Estanzuela 157/49" strain that was introduced from Uruguay as Plant Introduction Number 193144.

When seed inoculated with chlamydospores of *Ustilago bullata* was sown at Stoneville in the falls of 1953, 1954, and 1955, Lamont rescuegrass proved immune from collections of the head smut fungus from Alabama, Texas, Mississippi, and Georgia. It proved moderately susceptible to a collection from Louisiana that appears to be a different race of the fungus. Commercial, Texas 46, Chapel Hill, and a Georgia selection from Chapel Hill rescuegrass were susceptible to each of the 6 collections of the head smut fungus.

No statistically significant differences in forage production between Lamont, Chapel Hill, and the Georgia selection from Chapel Hill were obtained in harvests made when the grass was in the immature-seed stage in the springs of 1954 and 1956. Texas 46 and Prairie brome yielded significantly less than did Lamont in the second test, but this appeared to be due to their thinner stands.

In a test involving 6 paired plots, planted at Stoneville on November 10, 1953, with seed inoculated with *U. bullata*, the Lamont variety yielded 254 lb. more clean seed per acre than did Chapel Hill. This difference was statistically significant. The low yield of seed from the Chapel Hill variety of rescuegrass was due to a large number of smutted panicles.

Lamont rescuegrass consists largely of plants that are biennials or short-lived perennials; consequently, it should provide a longer grazing season during the second and subsequent years of the stand than during the year of establishment. If grown as a winter annual, its grazing season would be similar to that of other varieties of rescuegrass.

The Lamont variety of rescuegrass (*Bromus catharticus* Vahl.) is a selection from the "La Estanzuela 157/49" strain. Seed of this strain was obtained at "La Estanzuela," National Phytotechnic and Seed Nursery Institute in Uruguay, by Dr. O. S. Aamodt and was catalogued as Plant Introduction Number 193144. A small plot of this strain of rescuegrass was sown at the Delta Branch Experiment Station, Stoneville, Mississippi, on November 10, 1951. It made excellent vegetative growth and produced a heavy seed

[1] Accepted for publication February 5, 1957.
A report of cooperative work conducted by the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Delta Branch of the Mississippi Agricultural Experiment Station at Stoneville, Mississippi.
[2] Plant Pathologist, F.C.R.B., A.R.S., U.S.D.A., and Mississippi Agricultural Experiment Station. The writer is indebted to R. A. Kilpatrick for taking the photographs.

TABLE 1.—*Mean percentage of smutted panicles, expressed as degrees, obtained when seed of 5 varieties of rescuegrass was inoculated with spores of 6 collections of Ustilago bullata at Stoneville, Mississippi*

Variety of rescuegrass	Percentage[a] of smutted panicles where seed was inoculated with spores from indicated collection						
	Raymond, Miss.	Stoneville, Miss.	Auburn, Ala.	Baton Rouge, La.	Watkinsville, Ga.	College Station, Texas	Variety means[b]
				1954-55 test			
Commercial	77.1	79.5	72.5	69.1	61.5	82.7	72.4**
Chapel Hill	45.8	47.4	73.9	42.5	81.1	45.4	53.9**
Watkinsville Selection	63.3	65.6	75.3	50.8	78.5	54.9	63.4**
Texas 46	24.8	43.7	30.7	25.0	39.4	37.3	33.8**
Lamont	0.0	0.0	0.0	24.8	0.0	0.0	9.8
Collection means[c]	42.2	47.2	50.5	42.4	52.1	44.1	
				1955-56 test			
Commercial	76.8	60.9	58.4	60.9	39.9	54.8	57.7**
Chapel Hill	55.7	67.2	78.8	77.8	81.9	61.6	68.6**
Watkinsville Selection	55.7	53.5	82.1	76.7	82.5	68.5	67.3**
Texas 46	53.7	47.3	30.0	48.4	43.4	47.5	45.1**
Lamont	0.0	0.0	0.0	21.4	0.0	0.0	8.5
Collection means[c]	50.3	48.4	49.8	55.1	49.0	49.0	

[a] Each figure is the mean of 4 replicates, each consisting of a 9-foot row.
[b] Double asterisks mark the means that are significantly higher than that of Lamont at the 1 per cent level. LSD = 5.7 degrees (1954–55) and 8.1 degrees (1955–56).
[c] Differences between these means are not statistically significant. LSD (5%) = 15.5 degrees for each test, when based on the variety × collection mean square.
Coefficient of variation = 14 per cent in 1954–55 and 13 per cent in 1955–56.

crop in the spring of 1952, but no seed harvest was made that spring.

Many of the plants in this plot persisted throughout the hot, dry summer of 1952 and resumed active growth that fall. Volunteer seedlings also developed in the fall of 1952. Approximately ½ lb. of seed was stripped from the plants by hand on May 25, 1953. This was harvested chiefly from the 2-year-old plants and was the original seed of the selection that is now called Lamont.

HEAD SMUT RESISTANCE.—Seed of the Lamont and Chapel Hill varieties of rescuegrass was inoculated with chlamydospores of the head smut fungus (*Ustilago bullata* Berk.) in November, 1953. The spores used in the inoculations had been collected at Raymond, Mississippi, on Commercial rescuegrass in April, 1953.

In the preparation of the inoculum, smutted spikelets were placed in a mortar and ground with a pestle until a fine black powder of spores was obtained. This inoculum was placed in each seed packet and the packets shaken until the seed was heavily coated with spores. The inoculated seed was sown in 10 paired rows, each 10 ft. long, at Stoneville on November 10, 1953.

In the spring of 1954, the 10 rows of Chapel Hill rescuegrass from inoculated seed were heavily smutted, whereas the 10 rows of similarly inoculated Lamont rescuegrass were smut free. The panicles from approximatley 3 ft. of each row were harvested on May 20, 1954, and placed in large paper bags. Numbers of smutted and smut-free panicles were determined soon thereafter. From 30.0 to 62.6 (average 49.9 per cent) of the panicles from the 10 rows of Chapel Hill rescuegrass were smutted. In contrast, no smutted panicles were found in the harvest from the 10 rows of Lamont rescuegrass.[3]

A plot for a more extensive inoculation test was seeded at Stoneville on October 19, 1954. In addition to the 2 varieties of rescuegrass planted in 1953, the test included Commercial, Texas 46, and a selection from the Chapel Hill variety of rescuegrass made at Watkinsville, Georgia. Six collections of the head smut fungus, instead of the single collection tested in 1953, were used in the trial. The sources of the collections were as follows: P. B. Gibson, Auburn, Alabama; W. H. Stroube, Baton Rouge, Louisiana; E. C. Holt, College Station, Texas; B. H. Hendrickson, Watkinsville, Georgia; and Raymond and Stoneville, Mississippi (collected by the writer). The inoculum was prepared and placed in the seed packets as before. The planting was made in 4 randomized split-plot blocks, with the rescuegrass varieties in main plots and the fungus collections in sub-plots. Each sub-plot was a 9-ft. row.

In May, 1955, panicles were harvested at random from each row in the test and placed in large paper bags. Counts of the number of smutted and smut-free panicles were made soon after harvest. The number of panicles harvested varied from 65 to 310 per row but was usually between 100 and 200. The results of the counts were calculated as percentage of smutted panicles. The percentages were transformed to angles, or degrees, and the transformed data were subjected to the analysis of variance.[4]

The Commercial, Chapel Hill, and Texas 46 varieties and the Watkinsville selection from the Chapel

[3] Johnson, H. W. 1956. A head smut resistant strain of rescuegrass from Uruguay. (Abs.) Phytopathology 46: 16.
[4] Bliss, C. I. 1938. The transformation of percentages for use in the analysis of variance. Ohio J. Sci. 38: 9-12.

Hill variety were susceptible to each of the 6 collections of *U. bullata* (Table 1). In contrast, Lamont rescuegrass was immune from 5 of the fungus collections but was moderately susceptible to the collection from Baton Rouge. The Baton Rouge collection appears, therefore, to be a different race of the fungus. The means for the Commercial, Chapel Hill, and Texas 46 varieties and for the Watkinsville selection from Chapel Hill are all significantly higher than the mean for the Lamont variety. Comparative plants of Chapel Hill and Lamont rescuegrass from this inoculation test are shown in Figure 1.

The differences between the means for the 20 rows grown from seed inoculated with each fungus collection are not statistically significant; however, a comparison of the means of the different collections on a single variety reveals a number of differences that are large enough for statistical significance (LSD (5%) = 7.6). It is evident that in a test of this type the reaction of the individual varieties to the different collections of *U. bullata* is of greater importance than is a comparison based upon the means of the collections on the 5 varieties combined.

Plots for a similar inoculation test were sown at Stoneville on October 26, 1955. This test differed from the others only in that the inocula representing the 6 collections were all collected at Stoneville in the spring of 1955 on the Chapel Hill variety of rescuegrass. Since the Chapel Hill variety showed no smut in the noninoculated rows in the 1954–55 test, the possibility of mixture through seed-borne inoculum did not exist. There did remain the possibility of mixture through wind-blown spores during the spring of 1955. The results obtained in 1955–56 agreed so well with those obtained in 1954–55, however, that one seems justified in concluding that the different collections remained practically unchanged during the year they grew at Stoneville.

In May, 1956, panicles were harvested from each row, as had been done in 1955, and the percentages of smutted panicles were determined. The percentages were transformed to angles, or degrees, as before and the transformed data were subjected to the analysis of variance.

The Commercial, Chapel Hill, and Texas 46 varieties and the Watkinsville selection from Chapel Hill were susceptible to each of the 6 collections of *U. bullata* (Table 1), as was the case in 1954–55. Lamont rescuegrass again proved immune from 5 of the fungus collections but was moderately susceptible to the collection from Baton Rouge. The means for the Commercial, Chapel Hill, and Texas 46 varieties, and for the Watkinsville selection from Chapel Hill are all significantly higher than is the mean for the Lamont

FIG. 1.—Ends of 2 rows of rescuegrass in the 1954-55 test plots from seed inoculated with *Ustilago bullata*. Photographed on May 5, 1955, to show A) the preponderance of erect, lightweight, smutted panicles in Chapel Hill and B) the drooping, heavy, seed-filled panicles and complete freedom from head smut in Lamont.

variety. The means for collections differ only slightly when all varieties are combined, and these differences are not statistically significant. The means for collections on single varieties differed significantly, as in the previous test (LSD (5%) = 13.0).

The analyses of variance for the inoculation tests of 1954–55 and 1955–56 showed that the *F* values for varieties, collections, and the variety × collection interaction are all significant at the 1 per cent level each season. Thus, the odds are greater than 99 to 1 that the differences obtained were not due to chance.

FORAGE PRODUCTION.—The paired rows of Chapel Hill and Lamont rescuegrass remaining in the field after the panicles were harvested from the ends of the rows on May 20, 1954, were trimmed to 5 ft. in length. On June 1, green weights of forage were determined for each 5-ft. row. Shrinkage samples were taken from each of the 20 green samples and oven dried. The percentage of dry matter was determined from these samples and was used in calculating the yield of moisture-free hay per row and per acre.

The yield of moisture-free hay was greater for the Lamont variety than for Chapel Hill in 8 of the 10 pairs of rows. The mean yield for Lamont rescuegrass in tons per acre was 2.9 and that for Chapel Hill was 2.6; however, this difference was not statistically significant by the *t* test for paired samples.

Plots for a test were planted at Stoneville on October 24, 1955, to compare the forage yield of Lamont rescuegrass with that of the following 4 varieties: Chapel Hill, Watkinsville Selection, Texas 46, and Prairie brome. The test plots were sown in 4 randomized blocks with each plot consisting of 4 20-ft. rows. On May 4, 1956, green weights of forage were determined for 14 ft. of one of the 2 center rows of each plot. The percentage dry matter was obtained from shrinkage samples and used in calculating the yield of moisture-free hay per row and per acre. The mean yields in tons/acre were as follows: Chapel Hill, 4.9; Lamont, 4.8; Watkinsville Selection, 4.4; Texas 46, 3.1; and Prairie brome, 2.7.

Texas 46 and Prairie brome yielded less than did Lamont in this test, and the differences were statistically significant. The stands obtained in the rows of Texas 46 and Prairie brome were considerably thinner than the stands obtained in the rows of the other 3 varieties, and the lower yields appeared to be a result of the thinner stands. The differences between Chapel Hill, Watkinsville Selection, and Lamont were not significant by the analysis of variance.

SEED PRODUCTION.—To compare seed production of the Chapel Hill and Lamont varieties, 6 paired plots (each 5 × 5½ ft.) were sown with inoculated seed at Stoneville on November 10, 1953. In May, 1954, it was observed that approximately one-half the panicles in the Chapel Hill plots were smutted whereas the panicles in the Lamont plots were smut free. The panicles from each plot were harvested in June, 1954, and placed in large cloth bags. The seed was threshed and the weight of the cleaned seed obtained from each plot was determined.

The yield of cleaned seed was greater for the Lamont variety than for Chapel Hill in each of the 6 pairs of plots. The mean yield for Lamont rescuegrass in lb./acre was 979 and that for Chapel Hill was 725. This difference was highly significant by the *t* test for paired samples. The difference was due to the large number of smutted panicles in the plots of Chapel Hill rescuegrass grown from inoculated seed.

On September 30, 1954, a small block consisting of 15 rows, each 20 ft. long, was seeded with Lamont rescuegrass at Stoneville. On October 6, 1954, another block consisting of 16 similar rows was seeded at a different location in the same general area. The rows in each block were 2 ft. apart. Both blocks were isolated from other rescuegrass plantings to provide a preliminary seed increase of this head smut resistant variety. On June 16, 1955, the grass in these 2 blocks was cut by hand and the seed threshed. These plots yielded at the rate of 562 lb. of clean seed per acre. In addition, sufficient seed shattered before and at harvest to give a solid stand of seedlings between the rows in the fall of 1955.

On October 20, 1955, a field of approximately 1.5 acres was seeded with Lamont rescuegrass in rows 40 in. apart. Approximately 5 lb. of seed was used. The field was well isolated from other rescuegrass plantings on the Experiment Station grounds at Stoneville. In late May, 1956, this field was trimmed at the ends and sides, leaving a block that was 161 ft. wide and 325 ft. long. From this 52,325 sq. ft., (approximately 1.2 acres), 940 lb. of cleaned seed was threshed during the first week of June, 1956.

The seed yield obtained was, therefore, at the rate of 783 lb. per acre. In addition, a large amount of seed shattered before and at harvest, as in the previous year. The seed harvested was of excellent quality, having a purity of 97.75 per cent, a germination of 89.50 per cent, and a test weight of 17.4 lb.

Over 90 per cent of the plants in this field persisted throughout the hot, dry summer and resumed growth in the fall of 1956. The results obtained at Stoneville indicate that one should be able to seed this grass in rows in the fall, harvest a seed crop the following spring, and by means of the persisting plants and volunteer seedlings have a solid planting suitable for pasture the autumn following seeding. If nitrogen is applied to this planting in September or October, it should be possible to start grazing by late November or early December in most seasons. This would provide a much longer grazing season than is obtained when rescuegrass is grown as a winter annual.

FIELD CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE
AND
DELTA BRANCH, MISSISSIPPI AGRICULTURAL EXPERIMENT STATION
STONEVILLE, MISSISSIPPI

STUDIES ON BACTERIAL BLIGHT OF LIMA BEAN[1]

Maung Mya Thaung and J. C. Walker

SUMMARY

Pseudomonas syringae v. Hall was shown to be present commonly on lima bean seed grown in the United States west of the Continental Divide. It survived in the soil during a Wisconsin winter and throughout the following growing season. Infection was obtained readily in the greenhouse when lima bean plants were sprayed with an atomized suspension of the bacteria with and without a post-inoculation moist period. The isolates used were not pathogenic on red clover. The optimum continuous temperature for growth of the pathogen in culture and for disease development was 28° C. When inoculated plants were subjected to alternating high and low temperatures, disease severity was determined by the night temperature. In culture, growth of the organism increased with increase in level of nitrogen at each sucrose level used. An increase in growth also resulted from an increase in sucrose level, but this was less marked than with nitrogen. Disease development was markedly enhanced in plants grown in high-nitrogen, low-phosphorus, and low-potassium nutrients when compared with those grown in a balanced solution. A statistically significant but less pronounced increase in disease index occurred when plants were supplied with high-phosphorus or high-potassium nutrient.

Bacterial blight of lima bean (*Phaseolus limensis* Macf.) was first noted in 1892 in New York (1). It was reported by Halsted (8) from New Jersey and Pennsylvania in 1893 and by Sturgis (12) from Connecticut in 1899. The incitant was described by Tisdale and Williamson (13) as *Bacterium viridifaciens* in 1923. Earlier in the same year, Gardner and Kendrick (6) described the incitant of bacterial spot of cowpea (*Vigna sinensis* (L.) Endl.) as *Bacterium vignae*. They later showed *B. vignae* and *B. viridifaciens* to be identical (7). The bacterial blight pathogen on clover (*Trifolium* spp.) was described in 1923 as *Bacterium trifoliorum* by Jones et al (10). Elliott (4) regarded all of the above-mentioned pathogens as synonymous with *Pseudomonas syringae* v. Hall, which was described on lilac (*Syringa vulgaris* L.) in 1902 (14). She listed 75 species in 41 genera as host plants. The symptoms of the disease on lima bean were described by Tisdale and Williamson (13) and by Harter and Zaumeyer (9). The relation of *P. syringae* to lima bean tissue was described by Beach (2).

In Wisconsin, bacterial blight of lima bean appears in severe form spasmodically. In such cases, it usually causes a sudden, almost complete defoliation except for the youngest leaves. After such defoliation, the plants often continue to grow and may recover almost completely. In 1954, an epidemic appeared about 6 days after, and in the path of, a cyclonic storm in which wind velocity approached 100 miles per hour. The storm was followed promptly by a heavy rain. Within the epidemic area, the disease was most severe in crops growing on light sandy soil. A similar rapidly developing epidemic in Connecticut in 1898 was described by Sturgis (12).

Inasmuch as current information was insufficient to explain the source of inoculum and the rapid development of the epidemic, the present study was undertaken. The scope of this investigation includes the overwintering of the pathogen in seed and in soil, the relation of environal factors to the disease cycle, and the relation of host nutrition to disease development.

SOURCE OF CULTURES AND METHODS OF INOCULATION.—In the course of these studies, 4 isolates of *P. syringae* were used. Isolate W was isolated from diseased lima beans in Wisconsin. PS20 and PS21 were received from W. H. Burkholder, Cornell University. They were isolated from lilac and from pear blossoms, respectively. PS109 was received from M. P. Starr, University of California. It was isolated from lilac.

Single-cell isolates were derived from each culture according to the method of Lederberg (11). The original cultures and the single-cell isolates were maintained on nutrient-agar slants under mineral oil at 8° C. They were tested periodically for pathogenicity on lima beans and reisolated. They also were submitted from time to time to cultural tests to ascertain their identity and freedom from contamination.

Two methods of inoculation of lima bean plants were used. Unless otherwise indicated, the plants were exposed to moisture-saturated atmosphere in a chamber for 2 hours before inoculation and for 48 hours after inoculation. In certain experiments, inoculation was made on plants on the greenhouse bench without use of a moist chamber. This method is referred to herein as "inoculation *in situ*." In both types of inoculation, the inoculum was applied with an atomizer attached to a compressed air line at 20-lb. pressure. The first symptoms of the disease usually appeared 2–3 days later.

A standardized inoculum was used in the environal and nutritional studies. The organism was grown on nutrient agar for 48 hours at room temperature. The bacteria were scraped into sterile distilled water, and the optical density of the suspension was adjusted to a value of 3 with a Lumetron photoelectric colorimeter (Model No. 400-A) equipped with a red filter (650

[1]Accepted for publication February 9, 1957.

mμ).

One week after inoculation, the plants were examined for the extent of lesion development on the leaflets and placed in one of the following classes: 0 = no symptoms of infection; 25 = 1 to 5 lesions per leaflet; 50 = 6 to 50 lesions per leaflet; 75 = more than 50 lesions per leaflet; 100 = severe leaflet distortion or death. These classes are illustrated in Figure 1. The number of plants in each class was multiplied by the class number and the total of value for each treatment divided by the number of plants in the treatment to give the disease index. Thus, if all plants were healthy the disease index would be 0, whereas it would be 100 if all had severely distorted leaves or were dead.

SEED TRANSMISSION.—Almost all of the lima bean seed used in Wisconsin for the processing crop is grown in the United States west of the Continental Divide in a semiarid climate and under irrigation. It has been generally assumed (9) that the disease is rare or absent in those regions and that seed grown therein is free from the blight organism. Since the disease in Wisconsin was often found in crops growing in fields not previously planted to lima beans, the possibility of introduction on western seed was investigated. Accordingly, 23 commercial samples of western-grown seed were obtained from canners and seedsmen for assay.

About 100 seeds of each sample were planted in a tray containing autoclaved white silica sand. Water was then added. Seedlings 3–6 in. tall were produced in 7–10 days. The presence of *P. syringae* in a given sample of seed was determined in 2 ways. The first method consisted of collecting cotyledons from all plants and homogenizing them in a Waring Blendor for about 5 minutes. The homogenate was strained through 2 layers of cheesecloth and then through filter paper on a Buchner funnel. The filtrate was then tested on lima bean plants. In the second method, the primary leaves of the plants from which cotyledons were collected were examined for lesions. The presence of the pathogen in such lesions was confirmed by isolation and inoculation.

In 12 of the 13 samples in which positive infection was obtained with the cotyledon homogenate, lesions also were observed later on primary leaves (Table

TABLE 1.—*Assays of commercial seed lots of lima beans for Pseudomonas syringae*[a]

Lot no.	Presence of pathogen in homogenate[b]	Percentage of seedlings with lesions
1	+	27
2	—	0
3	—	0
4	+	21
5	—	0
6	+	25
7	+	25
8	—	0
9	—	0
10	—	0
11	+	12
12	—	0
13	+	24
14	+	23
15	—	0
16	—	19
17	+	36
18	+	8
19	+	44
20	+	9
21	+	21
22	—	0
23	—	0

[a] Approximately 100 plants per test were used in each of 2 tests.

[b] + = Organism present; — = organism absent. The homogenate was made by collecting cotyledons from seedlings and grinding in a Waring Blendor.

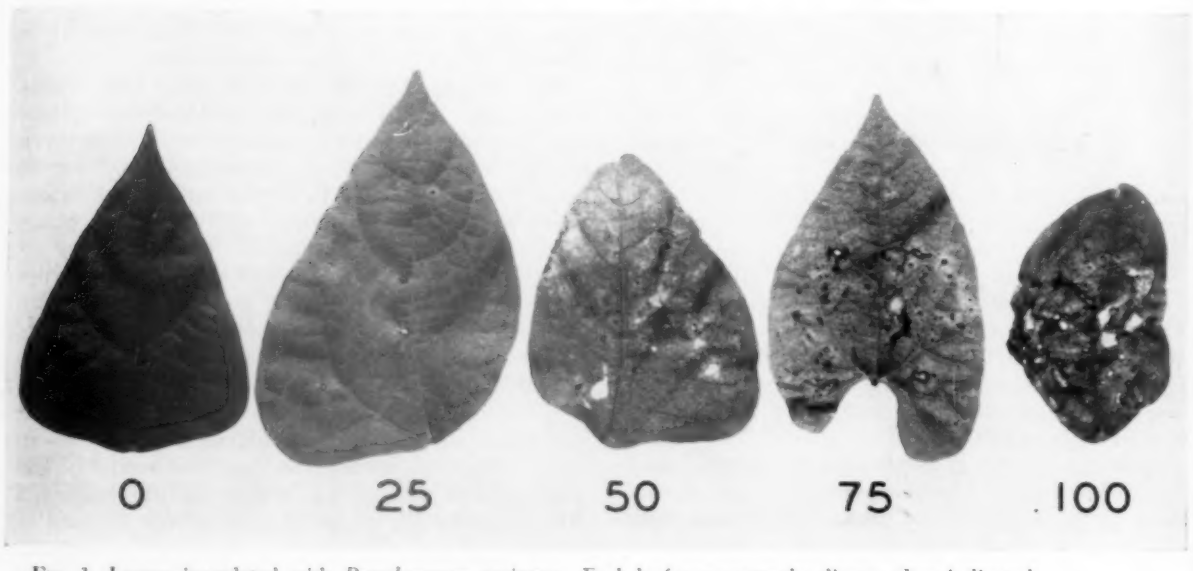


FIG. 1. Leaves inoculated with *Pseudomonas syringae*. Each leaf represents the disease class indicated.

1). In 1 sample, 19 per cent of the seedlings bore lesions but no infections resulted when the homogenate was tested on lima beans. Inasmuch as 13 of the 23 western seed samples assayed yielded positive evidence of transmission of the pathogen, it may be safely concluded that the organism is prevalent in western seed-growing areas, although possibly the disease is not conspicuous. It is evident also that infected seed is a common source of primary inoculum.

SOIL TRANSMISSION.—In a survey of Wisconsin lima bean fields in 1954, it was observed that the disease was usually most severe in fields that had grown 1 or more lima bean crops immediately previous to the current one. This indicated that soil also may be an important source of primary inoculum. A farm was selected in 1954 in which 2 or more successive crops of lima beans had been grown on several fields and in which the 1954 crop was severely diseased. Soil was collected from each of 5 fields in May, 1955; in August, 1955; and in May, 1956. Lima beans were grown in only 1 of these fields in 1955. The soil samples were assayed in 2 ways. A small amount of soil was agitated in sterilized distilled water and allowed to settle. A portion of the supernatant liquid was tested on lima bean plants. Another portion was used to seed plates of nutrient agar containing 0.1 per cent crystal violet. All bacterial colonies that resembled *P. syringae* were scraped off and suspended in sterile distilled water; the suspension was used to inoculate lima bean plants.

Each sample from the collections of May and August, 1955, gave positive infection. In the 1 field in which lima beans grew in 1955, very little evidence of disease was found. When the samples collected in May, 1956, were assayed, no infection was obtained. It is evident from these results that the organism lived over in Wisconsin soils for 1 year but not through 2 winters in the absence of the host.

LACK OF EVIDENCE OF OVERWINTERING ON RED CLOVER.—Jones et al (10) studied a bacterial blight of red clover (*Trifolium pratense* L.) and reported that an isolate of the incitant from Virginia was pathogenic on lima bean whereas one from Wisconsin was not. Clara (3) found an isolate from lima bean collected in Michigan, which was designated as *Bacterium vignae*, to be noninfectious on red clover. The 4 isolates used in this investigation were nonpathogenic on red clover. Moreover, no bacterial blight was observed on red clover in fields in the area where bacterial blight of lima beans was very destructive in 1954. Although Elliott (4) regarded the red clover blight organism as synonymous with *P. syringae* on the basis of cultural characteristics, there is no evidence that red clover is an overwintering host of the lima bean blight organism in Wisconsin.

RELATION OF TEMPERATURE TO BACTERIAL GROWTH AND DISEASE DEVELOPMENT.—Each isolate was grown on a nutrient-agar slant for 24 hours and then washed off with 10 ml of sterile distilled water. The resulting suspensions were diluted so that all had the same optical density. One ml of a suspension was sprayed from a syringe into a test tube containing 9 ml of nutrient broth of pH 7. The seeded tubes were placed in pairs at 4° intervals from 4° to 30° C for 36 hours, after which optical-density readings were taken. With all 4 isolates listed above, growth occurred at 16° to 36°, inclusive, but no change in density was observed in 36 hours at 12° and below. The maximum optical density was at 28°.

Progress of the disease at different temperatures was studied next. Plants were inoculated in the fifth week after sowing. Five pots containing 2 plants each were used per treatment. In the first 4 tests, plants grown at about 24° were inoculated and placed in a moist chamber at the same temperature for 2 days. An equal number of pots was then placed in greenhouse rooms held at 16°, 20°, 24°, and 28°. In the last 2 tests, plants were grown in greenhouse rooms at 16°, 20°, 24°, and 28° for 1 week, inoculated *in situ* and then held at the respective temperatures. Disease severity for each pot was recorded 1 week after inoculation, and a disease index for each treatment was calculated (Table 2). In every test, the disease was most severe at 28°. At any given temperature, the indices in tests in which moist treatment was given after inoculation (Tests 1 to 4, inclusive) and those of tests in which plants were inoculated *in situ* (Tests 5 and 6) did not differ appreciably.

Since growing crops are subject to fluctuating temperatures with maxima usually in the day and minima during the night, a series of experiments was next run in which blight development at alternating high and low temperatures within a 24-hour period were compared with that at continuous constant temperatures. The 16° and 28° temperatures were chosen. Four weeks after sowing, plants were placed for 1 week in 4 series: 1) 16° continuous; 2) 28° continuous, 3) 16° day and 28° night; 4) 28° day and 16° night. They were then inoculated *in situ* and continued in the same environments for 1 week, after which disease

TABLE 2.—*Severity of bacterial blight on lima bean plants that were held in greenhouse rooms at various temperatures for 1 week after inoculation* [a]

Holding temperature °C	Disease index [b] for the test and treatment indicated						
	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Average
16	40	40	36	45	42	45	41
20	51	43	40	39	48	44	44
24	51	45	51	41	51	48	48
28	69	61	65	66	68	70	67
LSD 5 per cent							4
1 per cent							6

[a] In tests 1 to 4, inclusive, plants grown at 24° were inoculated, held in a moist chamber at 24° for 48 hours, then placed at the indicated temperatures. In tests 5 and 6, the plants were held at the indicated temperatures before and after they were inoculated *in situ*.

[b] Based on a scale of 0 to 100, in which 0 indicates no symptoms and 100 indicates severe leaf distortion or death. Each index figure, except those in the average column, is based on class values of 10 plants.

severity was recorded. The length of day was regulated so that in all cases a 12-hour light period alternated with a 12-hour dark period.

As in the previous experiment, blight was much more severe on plants kept continuously at 28° than on those kept at 16° (Table 3). When alternating day and night temperatures were used, however, there was a wide difference in blight severity on plants subjected to the 2 alternating schedules. The temperature of the 12-hour dark period was that which determined the disease index. In both schedules, the day temperature had little or no effect on disease development.

CARBON-NITROGEN NUTRITION OF THE PATHOGEN IN CULTURE.—The basal medium contained 1.3 g KH_2PO_4, 1.2 g K_2HPO_4, and 0.3 g $MgSO_4 \cdot 7H_2O$ per liter of distilled water, with asparagine and sucrose added in various amounts as sources of nitrogen and carbohydrate, respectively. Each isolate was grown on a nutrient-agar slant at room temperature for 12 hours and then washed off with 50 ml of sterile distilled water. One ml of the suspension was sprayed into 50 ml of test medium contained in a 125-ml Erlenmeyer flask. Each treatment was made in duplicate. The flasks were placed in a shaker for 36 hours, after which period bacterial growth was estimated by optical-density readings. Since all isolates followed the same general trend of growth in each of 4 separate experiments, only those readings obtained with the Wisconsin isolate (W) are presented (Fig. 2).

Regardless of the level of sucrose, and even in its absence, bacterial growth increased progressively as the level of nitrogen was increased. Production of green pigment was independent of the sucrose supply: it was initiated by the addition of a minimum of about 0.01 per cent nitrogen to the basal medium, and its intensity increased with increase in the level of nitrogen. Whereas increases in sucrose tended to increase growth at any given nitrogen level, the influence of the sugar appeared to be much less than that of asparagine.

HOST NUTRITION IN RELATION TO DISEASE DEVELOPMENT.—Lima bean seeds were sown in silica sand, and 2 weeks later the seedlings were transplanted in pairs to sand in 7.5-in. glazed crocks. The crocks were fitted with siphon tubes by means of which excess nutrient solution was removed after application to the surface of the sand. Nutrient solution was applied weekly. In certain cases, plants were grown in sand in pots to which a continuous supply of nutrient was provided by a drip system described by Gallegly and Walker (5). Plants were grown in the respective nutrients for 3 weeks after transplanting and were then inoculated and placed in a moist chamber for 48 hours, except that those grown in the drip system were inoculated *in situ*. Disease readings were taken 1 week after inoculation.

TABLE 3.—*Severity of bacterial blight on lima bean plants held at constant temperatures and at fluctuating temperatures (12-hour cycle) for 1 week prior to inoculation in situ and for 1 week afterward*

Temperature °C	Disease index[a] for the test and treatment indicated					
	Test 1	Test 2	Test 3	Test 4	Test 5	Average
16° Continuous	52	50	57	52	54	53
28° Continuous	73	65	70	75	72	71
16° Day, 28° night	70	78	76	70	73	73
28° Day, 16° night	50	58	55	60	56	56
LSD 5 per cent						4
1 per cent						5

[a] Based on a scale of 0–100, in which 0 indicates no disease and 100 indicates severely distorted leaves or death. Each index figure, except those in the average column, is based on class values of 10 plants.

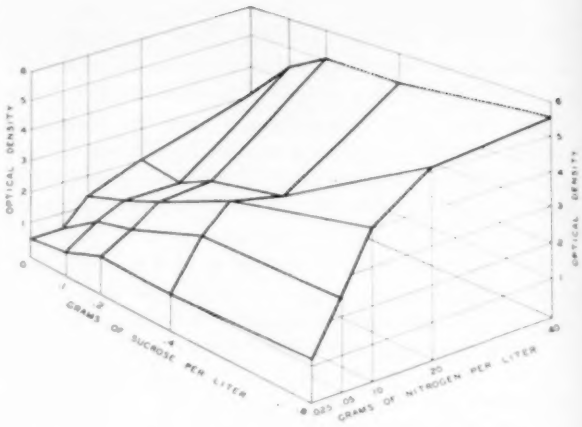


FIG. 2. Growth of *Pseudomonas syringae* in liquid media containing different levels of sucrose and of nitrogen. Relative amounts of growth were determined by optical-density measurements made at 650 mμ with a photoelectric colorimeter.

The basal nutrient solution (1 H) and the solutions high and low in nitrogen, phosphorus, or potassium were the same as those described by Gallegly and Walker (5). In the low-mineral solutions, the level of the element concerned was one-tenth that in the basal solution, whereas in the high-mineral solutions the level was 3 times that in basal solution. The results of 6 tests are summarized in Table 4.

As in the experiments reported in the temperature series, there were no appreciable differences in disease indices of experiments with the 2 types of inoculation. Statistically significant increases in index resulted from all treatments except low N. The treatments that resulted in markedly higher disease indices than did the basal nutrient were those of high N, low P, and low K.

DISCUSSION.—The purpose of this investigation was to determine what factors were responsible for the severe epidemics of bacterial blight that appeared spasmodically in lima bean fields in Wisconsin. Infected seed and infested soil were demonstrated to be sources of inoculum. Field observations indicated, however, that although inoculum can be provided by

TABLE 4.—*Severity of bacterial blight on lima bean plants grown at various levels of nitrogen, phosphorus, and potassium*

Nutrient solution[a]	Disease indices[b] for test and treatment indicated						
	Test 1	Test 2	Test 3	Test 4	Test 5[c]	Test 6[c]	Average
Basal (1H)	55	56	55	54	55	57	55
High N	76	65	71	79	72	74	72
Low N	53	55	56	59	56	58	56
High P	58	60	63	61	62	63	61
Low P	69	77	74	73	72	75	73
High K	56	57	59	62	59	61	59
Low K	78	71	69	71	72	74	72
LSD 5 per cent							3
1 per cent							4

[a] High levels were 3 times that in the basal medium, and low levels were one-tenth that in basal medium.

[b] Based on a scale of 0 to 100, in which 0 indicates no disease and 100 indicates severe leaf distortion or death. Each index, except for those in the average column, is based on the class values of 12 plants.

[c] Plants grown in continuous drip nutrient system and inoculated *in situ*. In other experiments, nutrient was supplied intermittently.

these 2 routes, epidemics do not occur except in the path of heavy wind storms. This suggests that windborne infested soil particles are major means of widespread dissemination of the organism.

The results of the temperature studies indicated that the progress of the disease in the field would be influenced more by the night than by the day temperature.

The growth of the organism in culture was influenced more by the level of asparagine than by that of sucrose. It was not surprising, therefore, to find that when the host plants were fed a high-nitrogen nutrient the disease index increased markedly over that of those fed a balanced nutrient. Insufficient data are available to explain the several nutritional effects noted. It is suggested, however, that the severity of an epidemic that develops when climatic conditions are especially favorable may be influenced somewhat by the fertility of the soil on which the crop is growing.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. ANON. 1892. Blight of lima beans. In some bean diseases. N. Y. (Geneva) Agr. Expt. Sta. Bull. 48, p. 331.
2. BEACH, W. S. 1928. The relation of Bacterium vignae to the tissues of lima bean. Penn. Agr. Expt. Sta. Bull. 226. 15 p.
3. CLARA, F. M. 1934. A comparative study of the green-fluorescent bacterial plant pathogens. Cornell Univ. Agr. Expt. Sta. Mem. No. 159, 36 p.
4. ELLIOTT, C. 1951. Manual of bacterial plant pathogens. Ed. 2. Chronica Botanica Co., Waltham, Mass. 186 p.
5. GALLEGLY, M. E., JR., AND J. C. WALKER. 1949. Plant nutrition in relation to disease development. V. Bacterial wilt of tomato. Am. J. Botany. **36**: 613-623.
6. GARDNER, M. W., AND J. B. KENDRICK. 1923. Bacterial spot of cowpea. Science **57**: 275.
7. GARDNER, M. W., AND J. B. KENDRICK. 1925. Bacterial spot of cowpea and lima bean. J. Agr. Research **31**: 841-863.
8. HALSTED, B. D. 1893. A bacterium of Phaseolus. New Jersey Agr. Expt. Sta. 13th Ann. Rept. **1892**: 283-285.
9. HARTER, L. L., AND W. J. ZAUMEYER. 1944. A monographic study of bean diseases and methods for their control. U. S. Dept. Agr. Tech. Bull. 868. 160 p.
10. JONES, L. R., M. M. WILLIAMSON, F. A. WOLF, AND L. MCCULLOCH. 1923. Bacterial leafspot of clovers. J. Agr. Research **25**: 471-490.
11. LEDERBERG, J. 1954. A simple method for isolating individual microbes. J. Bacteriol. **68**: 258-259.
12. STURGIS, W. C. 1899. A bacterial blight of lima beans. Connecticut Agr. Expt. Sta. 22nd Ann. Rept. **1898**: 262-263.
13. TISDALE, W. B., AND M. M. WILLIAMSON. 1923. Bacterial spot of lima bean. J. Agr. Research **25**: 141-154.
14. VAN HALL, C. J. J. 1902. Bijdragen tot de kennis der bakterieele plantenziekten. Dissertation, Amsterdam Univ. Coöperative Drukkerij-vereeniging "Plantijn." Amster. 198 p.

PRUNE CRINKLE LEAF [1]

Earle C. Blodgett [2]

SUMMARY

Observations and transmission tests over a period of many years have shown that prune crinkle leaf, common on Italian Prune, is bud perpetuated but is not caused by an agent transmissible by budding. It is concluded that this disorder, as well as the somewhat similar sparse leaf, is due to genetic instability. Although prune crinkle leaf resembles cherry crinkle leaf of the Bing variety of sweet cherry, in appearance and behavior, the two are regarded as different.

A peculiar leaf disorder has been observed on Italian Prune (*Prunus domestica* L.) for many years. The symptoms are similar to those of sweet cherry crinkle leaf (6), and many times it has been designated informally as "crinkle." The present paper summarizes the current information and suggests the name "prune crinkle leaf" for the disorder.

DISTRIBUTION.—The writer has observed prune crinkle leaf over a period of about 20 years in orchards of Italian Prune of all ages and also in nursery rows. It has been noted in Idaho, Oregon, Washington, and Utah and probably occurs wherever Italian Prunes are grown.

SYMPTOMS.—Leaves on crinkle-leaf-affected trees show a wide range of leaf deformity, the most common of which are uneven margins and distorted blades (Fig. 1). Affected leaves generally are smaller and lighter in color than normal and often show chlorotic areas near the restricted portion of the blade. The mottled or marbled appearance is dull and quite distinct from the even green coloring of normal leaves. In older trees, prune crinkle leaf symptoms may occur only on 1 spur, branch, or larger portion of the tree. These frequent occurrences on formerly healthy branches probably represent genetic instability. The symptoms and characteristics are quite similar to those described for sweet cherry crinkle leaf, which occurs most commonly on the Bing and Black Tartarian varieties of sweet cherry. Trees with prune crinkle leaf usually are dwarfed; the growth is spindly, and the fruit may ripen earlier than normal. In general, crinkle-affected-trees tend to be unfruitful, although a fair crop may set, much of it to fall before maturity. The fruit stems on affected trees are noticeably shorter than those on normal trees. There are cases, however, in which trees with prune crinkle leaf are extremely vigorous but essentially unproductive, a condition often found in sweet cherry affected with crinkle leaf. Examples are known in which normal branches developed on crinkle-affected prune trees.

VARIETIES AFFECTED.—The writer has observed prune crinkle leaf in Italian Prune, in Early Italian Prune (Demaris), and in Greata, an early type Italian Prune grown in British Columbia, Canada. According to L. C. Cochran (personal correspondence), crinkle leaf symptoms have been seen on Emily, Hungarian Prune, and 1 or 2 more types of *P. domestica*. Stanley Prune also may exhibit symptoms, but the extremely vigorous growth may account for false symptoms just as is well known in the case of sweet cherry.

In many selections of trees affected with prune crinkle leaf, other somewhat similar troubles are also present and may be very confusing. Some of these are mottle leaf—cause unknown (2); necrotic leaf spot—caused by genetic factors (4, 1); chlorosis, often accompanying leaf spot; and a yellow-leaf and defoliation condition—cause unknown. The ring spot virus is commonly present in crinkle-leaf-affected Italian Prune trees as shown by indexing on Shiro-fugen flowering cherry. Prune dwarf (5), a virus disease, is characterized by leaf deformities that are distinct from those of crinkle leaf.

Italian Prune trees affected with the disease called sparse leaf (3) bear foliage somewhat similar to that on crinkle-leaf-affected trees; however, the type specimens of sparse-leaf-affected trees are robust growers of a determinate type; this gives the appearance of an umbrella shape, as contrasted to the dwarfed dense foliage of crinkle-leaf-affected trees. Trees with sparse leaf also leaf out and bloom about 10 days later than do normal or crinkle-leaf-affected trees.

TRANSMISSION TESTS.—During the course of investigations of stone fruit diseases since 1936 both in Idaho and Washington, many attempts to transmit an agent that induces prune crinkle leaf have been made but without success. Observations of budwood source trees and of nursery stock propagated from them show that prune crinkle leaf is readily perpetuated. Crinkle leaf symptoms have been noted on a small percentage of the seedlings grown from Italian Prune seeds obtained from a commerical cannery. Seedling foliage varied from that typical for Italian Prunes to foliage with extreme abnormalities, including lethal chlorosis or albinism. Seed transmission of factors causing crinkle leaf and other abnormalities is well known in sweet cherry.

In March, 1952, a grower in the Yakima Valley of Washington planned to remove a large prune tree that for many years had shown odd-shaped leaves and light crops. The entire tree was affected by crinkle leaf and had grown to maturity among normal trees

[1] Accepted for publication February 12, 1957.
Published as Scientific Paper No. 1512, Washington Agricultural Experiment Stations.

[2] Plant Pathologist, Washington State College and Washington State Department of Agriculture, Irrigation Experiment Station, Prosser, Washington.

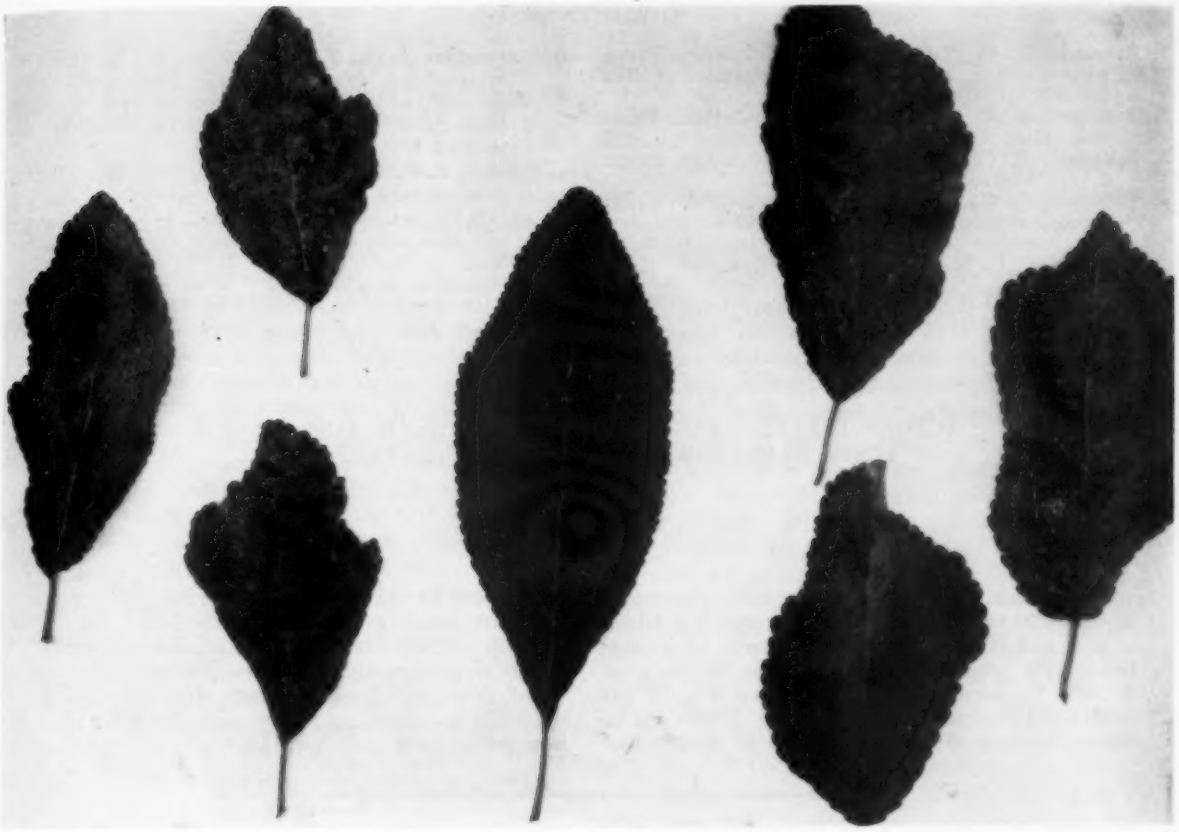


FIG. 1. Six Italian Prune leaves affected with prune crinkle leaf. Note irregular margins, marbled appearance, and chlorotic areas. Healthy leaf in center.

in the orchard. The grower was persuaded to leave the tree for transmission tests. The tree was dehorned, and approximately 20 branches $\frac{3}{4}$ to 2 in. in diameter were topworked with dormant scions from PR-H-1, a selected clone of healthy Italian Prune. Union was good in nearly every case, and the resulting growth from the healthy scions was extremely vigorous. During the 3 succeeding seasons, all leaves on the shoots from the scions were normal, whereas the remainder of the tree continued to show crinkle leaf symptoms. Late in 1954 the whole prune orchard was removed.

A similar test under the writer's guidance was performed by a grower near Walla Walla, Washington, in 1952. Six 4-year-old Italian Prune trees that had shown crinkle leaf since they were set in the orchard were top worked with healthy Italian Prune scions of clone PR-H-1. The unions were good, and growth from the scions the next 4 seasons had normal foliage.

DISCUSSION AND CONCLUSIONS.—Crinkle leaf symptoms on leaves of Italian Prune are of the same type that occur on crinkle-leaf-affected sweet cherry. Fruits on affected Italian Prune are not misshappen as on sweet cherry, but affected prune trees are sparse bearers like affected cherries. Healthy prune trees may "break down" and produce branches showing crinkle leaf symptoms just as do cherries. In both species there is evidence of seed transmission of a causal factor, and in both the disorder is easily perpetuated by grafting but there is no evidence of a graft-transmissible factor. Even with these striking similarities, there is no known relation between the 2 diseases.

Sparse leaf is the only other prune disorder that produces leaf symptoms similar to those of crinkle leaf. The difference in type of growth of sparse-leaf- and crinkle-leaf-affected trees seems to justify separating the disorders. Among the original sparse-leaf-affected trees, there are at least 2 trees that exhibit "reversion" of a branch to a healthy type of growth. This is in a prominent portion of the upper parts of the trees, and the occurrence is evidence that sparse leaf is noninfectious.

All the transmission trials with prune crinkle leaf support the theory that this disorder is genetic in nature and similar in this respect to sparse leaf and Italian Prune leaf spot.

IRRIGATION EXPERIMENT STATION
PROSSER, WASHINGTON

LITERATURE CITED

1. Blodgett, E. C. 1940. A leaf spot of Italian Prune perpetuated in budded stock. Phytopathology **30**: 347-348.
2. Blodgett, E. C. 1943. Prune diseases. Proc. Wash. State Hort. Assoc. **39**: 59-64.
3. Blodgett, E. C. 1947. Sparse leaf of Italian Prune. (Abs.) Phytopathology **37**: 360.
4. Blodgett, E. C., and B. L. Richards. 1951. Italian Prune leaf spot. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dep. Agr., Agr. Handbook 10, p. 210-211.
5. Hildebrand, E. M., T. B. Lott, and R. S. Willison. 1951. Prune dwarf. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr. Agr. Handbook 10, p. 171-174.
6. Kinman, C. F., W. B. Hewitt, T. B. Lott, G. L. Philp, E. L. Reeves, R. S. Willison, and S. M. Zeller. 1951. Sweet cherry crinkle leaf. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dep. Agr. Agr. Handbook 10, p. 195-200.

FACTORS INFLUENCING THE ELIMINATION OF LOOSE SMUT FROM BARLEY BY WATER-SOAK TREATMENTS [1]

L. E. Tyner [2]

SUMMARY

Loose smut in barley was effectively eliminated when 20–40 per cent by weight of water was added to seed and this treatment was followed by incubation of the seed in sealed flasks for 48 hours at 70°–75° F. Smut was not controlled if 5–15 per cent water was added to the seed. Smut was as effectively controlled by immersing the seed in running water for 54 hours as by soaking it for 54 hours in standing water, an indication that the agent or condition responsible for eliminating the fungus is present within the seed. Water in which wheat germ was fermented was markedly phytotoxic and fungitoxic, and loose smut was eliminated from barley seed soaked in this water for 20 hours.

Loose smut in barley and wheat may be effectively controlled by any one of several methods. For many years the hot-water treatment was the only known means of control. This method requires an initial soak period of several hours in unheated water followed by a short dip in hot water. Other methods of control that do not employ hot water have recently been established. The seed may be soaked in a water suspension of a fungicidal chemical (8), in water at room temperature for long periods (7, 8, 9), or placed under anaerobic conditions following a soak period of a few hours (3). Involved in all methods, however, is a period of immersion in water of short or of long duration. The absorbed water not only initiates the germination processes of the seed but also may activate the vital functions of the fungus mycelium within the embryo. With all methods of control, it appears that the smut fungus is more vulnerable to injury than is the embryo of the seed. In the hot-water method, heat is the lethal agent; in the other methods, the factors responsible for killing the smut fungus have not been determined. The present paper considers some of the possible factors.

Materials and methods.—Barley seed naturally infected with *Ustilago nuda* (Jens.) Rost. in the field in 1954 or 1955 was used. The seed was treated at room temperature (70°–75°F) in several ways: 1) seed to which 5, 10, 15, 20, 25, 30, 35, or 40 per cent by weight of water was added was sealed in flasks for 48 hours; 2) seed was soaked in water for 0.5 to 6 hours, then drained and sealed in pliofilm bags for 44–72 hours; 3) seed was soaked for 54 hours in standing water, in running water, or in water drained and replaced every 6–10 hours; and 4) seed was soaked in extracts of wheat germ for periods of 16 or 20 hours. After treatment, the seed was dried and four 12-ft. rows were planted, each with 200 seeds.

Additionally, various techniques were utilized to determine the effects of treatments on the barley seeds. Two samples of 22 seeds (1-g dry-weight basis) were taken from the flasks immediately after incubation with 5–40 per cent water in sealed flasks as in treatment 1 above. Oxygen consumption was determined in the Warburg apparatus over a period of 1 hour at 20°C. with air used as the gaseous phase. Rate of absorption of water by barley seed was determined by weighing blotted samples after various periods of immersion.

Results.—*Effect of moisture content of the seed.*—Water in various amounts was added to 50-g samples of Glacier barley in 250-ml flasks. The sealed flasks were incubated at room temperature for 48 hours, a period some hours less than that recommended for

[1] Accepted for publication February 19, 1957.
Contribution No. 1588 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] Officer in Charge, Plant Pathology Laboratory, Edmonton, Alberta.

control of loose smut (9). The intention was to obtain less than perfect control in certain treatments, thereby establishing a differential among them. Respiratory gases were determined on 1-g samples of seed from the flasks immediately after incubation.

The amount of smut in plants grown from seed to which 5, 10, or 15 per cent water had been added was not much less than the amount in plants grown from untreated seed (Table 1). A striking improvement in smut control, however, was evident in the seed to which 20 per cent water was added. In the remaining treatments, control of smut was almost complete; it is probable that the smut would have been completely eliminated by these treatments if the period in the sealed flasks had been extended for an additional 16 hours. It appears that 20 per cent is the minimum amount of added water necessary to initiate the processes leading to the control of smut. Although there was no visible evidence of germination in the seed in any treatment, it seems probable that the physiological processes of germination were activated in those receiving 20 per cent or more of water. It seems, therefore, that the conditions that cause the elimination of smut are established by the initial processes of germination in the infected seed when free oxygen has been depleted by the respiring seed.

The data on respiration of seed immediately following the incubation period indicate that oxygen consumption was low in the samples to which 5, 10, or 15 per cent water was added but that it increased markedly in the samples that received 20 per cent or more of water. Carbon dioxide evolution also was determined; the respiratory quotients were generally greater than 1, but it was not known whether the excess of CO_2 over O_2 was due to fermentation being operative or whether a Pasteur effect was involved (5).

It is of interest to report the results of further experiments related to the above. Glacier barley was soaked in water for periods of 0.5, 1, 1.5, 3, and 6 hours; it was then drained, sealed in large pliofilm bags for various times, dried, and planted in the field. Control of loose smut was good but incomplete in any treatment where the soak period was followed by 44, 46, or 48 hours in the pliofilm bags. The smut was completely eliminated, however, after 72 hours in the bags when the seed had been soaked for 3 or 6 hours. The amount of water absorbed by the seed during the 5 soak periods mentioned was 14, 16.6, 18.2, 23.4, and 30.5 per cent, respectively.

TABLE 1.—*Percentage of smutted heads on barley plants grown from seed[a] incubated at various moisture levels for 48 hours in sealed flasks at room temperature and oxygen uptake of the seed during 1 hour immediately following incubation*

Percentage water by weight	Average no. plants per row	Average no. smutted heads per row	μl Oxygen used per mg seeds per hour
0	145	29.3	
5	132	27.0	0.0027
10	117	27.0	0.0032
15	122	23.5	0.0027
20	120	4.5	0.0103
25	116	1.2	0.0166
30	100	1.0	0.0283
35	133	1.2	0.0575
40	117	0.0	0.0388

[a] Seed was naturally infected by *Ustilago nuda*.

Effect of renewal of water used to soak seed.—It has been shown that loose smut can be eliminated from barley by soaking it for extended periods in water at room temperature. Possible explanations of this effect have been proposed by Leben et al (4), who identified several organic acids in water in which barley, oats, or wheat had been soaked and showed that these inhibited the germination of spores of *U. nuda* at low pH levels. An inhibitory effect of anaerobiosis on *U. nuda* also was demonstrated.

The results of the following experiment should be considered in this connection. Comparable lots of Montcalm barley seed infected with *U. nuda* were soaked in water for 54 hours; soaked for 54 hours in water that was drained and replaced with fresh water at 8 a.m., 4 p.m., and 10 p.m. daily; or soaked for 54 hours in running water. The stand was good in all treatments, and the average number of smutted heads per row was 1.5, 0.7, and 0.2, respectively. In the control, there were 30 smutted heads per row.

Complete control of smut was not effected nor was it expected, because of the relatively short period of immersion. It is significant, however, that the smut was effectively controlled by running water. In this treatment, there would be no possibility that control of smut was accomplished through the accumulation in the water of microorganisms or their by-products or of products of respiration.

It seems that a toxic principle is formed within the seed and is operative there. In view of the above results, it is unlikely that it originates from the activities of microorganisms. It may be a chemical that is formed within the seed when germination is initiated under oxygen deficiency or it may be that the smut is eliminated as a result of prolonged reduction of oxygen tension.

Effect of by-products of fermentation within the seed.—Chloranil (tetrachloro-*p*-benzoquinone) is effective in eliminating loose smut from barley and wheat (7, 8, 9). The addition of this chemical to the steep solution rids the seed of the fungus in about 24 hours less time than when water alone is used. It has been established that quinones are formed in wheat and barley embryos when they are fermented (1, 2). Many quinones are fungicidal (6), and it was considered possible that the quinones that are formed in fermented seed are the agents responsible for killing the loose smut fungus within the embryo.

In further experiments, the water in which commercial wheat germ meal had been steeped for 2 days at 70°–75°F was used for the treatment of barley seed for the control of loose smut. One hundred g of wheat germ was added to 2 liters tap water and incubated. After incubation, the liquor was used without steriliza-

tion, after being sterilized by steam at 15 lb. for 20 minutes, and after it had been passed through a Seitz bacterial filter. Glacier barley seed was immersed in the solutions for 16 or 20 hours, dried, and then planted in field plots.

The solution that was not sterilized or filtered was extremely phytotoxic, whereas the autoclaved and the filtered solutions were only slightly so. This suggests that microorganisms were in some measure responsible for the phytotoxicity or that the toxic factor was partially removed by autoclaving or absorption on the filter. Effective control of loose smut was obtained by soaking the infected seed for 16 or 20 hours in the filtered solution and by immersing the seed in the sterilized solution for 20 hours (Table 2).

No significant control of loose smut can be achieved by soaking infected seed in water or by sealing moistened seed in air-tight containers for such short periods of 16 or 20 hours. It appears then that a highly fungitoxic material was produced by the fermenting wheat germ. Quinones were shown to be present in the solution, and there is a possibility that these may be the effective agents.

Discussion.—Several ways of controlling loose smut in barley and wheat have been developed in addition to the hot-water method. The seed may be soaked for 64 hours in standing or running water at room temperature; immersed for 40–44 hours in water containing chloranil; or soaked in water for 3–6 hours, drained, and placed for 2–3 days in pliofilm bags or other vessels that can be tightly sealed to exclude air. The factors that are common to these methods are imbibition by the seed of 20 per cent or more by weight of water and the maintenance of anaerobic or near anaerobic conditions during the course of the treatment. It has been suggested (4) that the organic acids formed in the steeping solution may play a part in destroying the loose smut fungus. The fact that the fungus was successfully controlled in running water or in water that was changed frequently during the course of the treatment makes it difficult to attribute great importance to compounds that are formed in the steep solution itself or to respiratory gases that diffuse into the medium. The present author has found organic acids quite phytotoxic. Barley seed did not survive when immersed in 0.25 per cent solution of acetic acid (pH 3.3) for 24 hours. Furthermore, no barley seed survived immersion in 5 per cent ethyl alcohol for 48 hours. It was found (unreported data) that smut can be eliminated from barley without an effect on the germinability of the infected seed. Thus, it may be presumed that the active agent or condition can be fungitoxic without being phytotoxic. It is most likely, however, that the fungus is more susceptible than is the host and mortality of either depends upon the concentration or intensity of the active agent or condition. Since a certain amount of water is necessary, it seems probable that the initiation of the physiological processes that accompany germination is a prerequisite. It is probable that some compound is formed or condition established during incubation under anaerobic conditions that kills the fungus mycelium in the embryo, and it is also probable that this occurs within the embryo itself.

Quinones have been identified in the fermenting germ of wheat or barley, and the extract from fermented wheat germ was found to be an effective agent in eliminating loose smut from barley seed. Chloranil has also been shown to be effective in controlling loose smut (7, 8, 9), and other quinones possess fungicidal properties (6). It is possible that quinones that are formed in the germ of wheat or barley during fermentation may be the agents that are responsible for the elimination of the fungus mycelium in the seed.

Table 2.—*Percentage of smutted heads on barley plants grown from seed[a] soaked at room temperature for 16 or 20 hours in unsterilized, filtered, and sterilized steep liquor obtained by fermenting wheat germ in water for 2 days*

Treatment of steep liquor[b]	Time (hours)	Average no. of plants per row	Average no. of smutted heads per row
Unsterilized	16	1.5	0
Autoclaved	16	82	3.7
Filtered	16	65	0.7
Unsterilized	20	0	0
Autoclaved	20	103	0.5
Filtered	20	92	0
Control (no treatment)		141	18

[a] Seed was naturally infected by *Ustilago nuda*.
[b] Autoclaving was at 15 lb. pressure for 20 minutes; filtration was through a Seitz filter.

Plant Pathology Laboratory
Science Service, Canada Department of Agriculture
Edmonton, Alberta

Literature Cited

1. Bungenberg de Jong, H. L., W. L. Klaar, and J. A. Vliegenthart. 1953. A procursor of methoxy-para-benzoquinone in wheat germ. Nature **172**: 402-403.
2. Cosgrove, D. J., D. G. H. Daniels, E. N. Greer, J. B. Hutchinson, T. Moran, and J. K. Whitehead. 1952. Isolation of methoxy- and 2:6-dimethoxy-p-benzoquinone from fermented wheat germ. Nature **169**: 966-967.
3. Hebert, T. T. 1955. A new method of controlling loose smut of barley. Plant Disease Reptr. **39**: 20-22.
4. Leben, C., R. W. Scott, and D. C. Arny. 1956. On the nature of the mechanism of the water-soak method for controlling diseases incited by certain seed-borne pathogens. Phytopathology **46**: 273-276.
5. Merry, J., and D. R. Goddard. 1941. A respiratory study of barley grain and seedlings. Proc. Rochester Acad. Sci. **8**: 28-44.
6. McNew, G. L., and H. P. Burchfield. 1949. Fungitoxicity and biological activity of quinones. Contribs. Boyce Thompson Inst. **16**: 357-374.
7. Russell, R. C., and L. E. Tyner. 1954. The influence of temperature on the time required to control loose smut of barley by means of the Spergon or water-soak treatments. Can. J. Agr. Sci. **34**: 533-538.
8. Tyner, L. E. 1951. Control of loose smut of barley by chemical and physical treatments. Sci. Agr. **31**: 187-192.
9. Tyner, L. E. 1953. The control of loose smut of barley and wheat by Spergon and by soaking in water at room temperature. Phytopathology **43**: 313-316.

FURTHER STUDY OF THE PRESERVATION OF PUCCINIA UREDOSPORES [1]

Eugene L. Sharp [2] and Frederick G. Smith

SUMMARY

Results of a study of uredospore behavior during drying and assay and over storage periods up to 5½ years are presented, mainly for *Puccinia coronata* and *P. graminis* f. sp. *avenae*. The essential features of the method are reduction of spore water content by high-vacuum drying to about 2 per cent (over-dry basis) and storage in absence of oxygen and water vapor. Quick freezing before evacuation and hygroscopic suspending media were not required. There was no conclusive evidence that differences in viability loss in drying or storage were related to extent or rate of drying or to drying method. There was some evidence, however, that storage temperature affected longevity. Slow rehydration of dried spores proved necessary for maximum germination and infectivity. Considerable variation was found in the storage life of the more than 40 batches processed from 20 spore collections. Dried spores from many collections have shown no decline in viability during periods ranging from 24 to 65 months. Dried batches from several greenhouse collections, however, have declined in viability during storage for 24–30 months. It seems likely that conditions of spore production may affect storage life of lyophilized spores as well as of those in cold storage. In general, the results indicate that the former can be expected to store at least 4 times as long as the latter. Lyophilized spores have proved to be effective when used for inoculation by several methods, and no evidence of change in infection type has been observed after drying and storage.

Because of the relatively short life, even in cold storage, of uredospores of the genus *Puccinia* (5, 7, 10) and because of the obligate nature of these pathogens, the maintenance and exchange of collections have been laborious and hazardous. In a previous report (12), drying of spores by lyophilization and storage in vacuum was shown to hold some promise of solution to this problem. The present report contains a more complete description of spore behavior during drying and assay and over storage periods up to 5½ years. The study was concerned primarily with *P. coronata* Cda. f. sp. *avenae* Eriks. & E. Henn. but includes some work with 3 other *Puccinia* species. The results presented are for spores of *P. coronata* unless otherwise stated.

MATERIALS AND METHODS.—The uredospores were collected from field or greenhouse host plants in or near Ames, except for those of *P. graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., which were greenhouse-produced spores sent from Kansas and Florida. The spores were stored in loosely stoppered tubes at about 5°C and 25–50 per cent relative humidity until processed.

Lyophil apparatus.—The lyophil apparatus was similar to those in general use (4), particularly that of Raper and Alexander (9). The smaller form first used consisted of rubber-connected glass primary and secondary manifolds with 20 outlets for lyophil tubes connected to a Cenco Hi-Vac pump through a cold trap containing solid CO_2 and acetone held at least 20°C below the temperature of the lyophil tube bath (method F, see below) or at less than −20°C in method NF (see below). In the larger form used after freezing prior to lyophilization was omitted (method NF), an 80-tube manifold was connected to the pump through a chemical desiccant trap (F. J. Stokes, Model 101) containing anhydrous $CaSO_4$ and silica gel. A McLeod gauge and spark coil were used to measure vacuum and to detect leaks.

Lyophil process.—1) Method F.—In most earlier experiments (1950-51), uredospores were dried by the conventional lyophil method (12), except that no suspending medium was used. Lyophil tubes attached to the manifold were immersed in a mixture of solid CO_2 and acetone at −45 to −50°C, and after about 2 minutes the vacuum pump was started. During the first drying period, the bath temperature was either brought slowly from −45° to −10° over a 2-hour period or was brought immediately to −10° and held there. During the second drying period of about 1 hour, the temperature was allowed to rise to room temperature. Several variations on this general pattern also were used. Finally, the individual tubes were sealed under vacuum before the pump was stopped.

2) Method NF.—In a few of the earlier experiments and in most of those since 1951, the spores were not frozen, and the entire drying operation period of 1–3 hours was carried out with tubes at room temperature. Since the spores presented a very large surface and were well insulated by the vacuum, it is possible that rapid freezing occurred because of evaporation of water; however, attempts to demonstrate this with small thermocouples immersed in the spore mass were not successful.

Spore samples in the individual 8-mm lyophil tubes ranged from 5 to 25 mg. Samples up to 500 mg were dried in 16-mm tubes of about 10-ml volume. The pressure of the vacuums ranged from 10 to 250 μ of Hg

[1] Accepted for publication February 19, 1957.

Journal Paper No. J-3098 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1258. These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and Iowa State College, NR 131-109.

[2] Present address: C Division, Chemical Corps Biological Laboratories, Fort Detrick, Frederick, Maryland.

in individual experiments, depending on the condition of the apparatus and the amounts of spores being dried, but most lots were dried at pressures of 50–100 μ. In the study of drying rate, the ordinary tubes were replaced by tubes containing a large-bore stopcock so they could be closed and removed for periodic weighing.

Viability and pathogenicity tests.—Viability was measured by germination counts in small petri dishes containing 3 per cent gelatin. Immediately after solidification of the medium, spores were dusted on from a camel's-hair brush, and the dishes were incubated 12–16 hours at 20°C. In most assays, a total of about 250 spores were counted for each sample in 5 fields in each of 2 dishes. Chi-square tests for representative samples indicated that deviations in germination counts between replicate fields were due to sampling errors, and probability values obtained indicated a binomial distribution of replicate field counts. For the number of spores counted, a difference of 12 in percentage germination was significant at the 5 per cent level (6, 13). Determination of standard deviation of differences between duplicate tube-stored samples also indicated that a difference of 12–14 per cent was significant at the 5 per cent level.

Infectivity tests were made in the greenhouse on primary leaves of Markton oat (*Avena sativa* L.) seedlings. Leaves were debloomed by rubbing unless otherwise specified, and spores were applied in most cases by brushing or spraying about 1 mg of spores suspended in distilled water onto each pot of 6 seedlings. Inoculated plants were placed in a moist chamber at about 21°C for 24–48 hours, and infection readings were taken after about 10 days in a 25° greenhouse by the method of Rosen and Weetman (10). For quantitative purposes, all pustules were counted on 10 leaves taken at random, and the leaf area was measured. Tests for stability of infection types were made by inoculation of selected members of the standard oat differential varieties (8).

Results.—*Survival during processing and storage.*—In the evaluation of the behavior of spores during processing and storage, periodic germination assays were made on over 50 lyophil batches from 20 spore collections. In the summary of these data (Table 1), only 1–3 assays of representative batches have been included; these are sufficient to show the rate and extent of deterioration where present.

The earlier evidence (12) that drying method NF gives somewhat higher survival during processing than does method F was confirmed (Table 1: Batches 6 vs. 7 and 12 vs. 13). This observation, plus the greater convenience of the former technique and the fact that no difference in storage behavior was observed during the first 2 years, led to the adoption of method NF as the standard technique since 1951. This practice seems to have been justified, since no consistent differences in storage life due to drying method have been observed to date (Table 1: Batches 6 vs. 7, 9 vs. 10, and 11 vs. 12).

Loss in germinability during the drying process itself has varied among batches and collections from 0 to 50 per cent, averaging about 20 per cent. Several factors appear to be involved in this variability. In addition to the effect of processing method, there is some evidence that for a given collection the loss was greater with spores that were fresh or high in germinability than after the germination had declined, probably because some of the weaker spores had already been eliminated. For example, several collections held in cold storage for periods of 3–5 months have shown smaller losses in drying than they did soon after harvesting. It is also likely, though not clearly established, that there were inherent differences in susceptibility to damage during drying that may depend on the conditions under which the spores were produced.

At the time of the first report (12) on the 1950 collections, no significant loss of viability in storage had been observed for periods up to 18 months. Since then, observations have been made on a much larger number of batches and spore collections over storage periods of from 24 to 65 months. The best storage life has been observed with batches from the field collections, II and VI. About one half of these batches still showed no significant loss of germinability after 65 months for II and 40 months for VI. The greenhouse collections I, III, and V also had not changed significantly when the last tubes available were assayed after 24–36 months' storage; however, all other greenhouse collections, VII to XIV, have decreased in viability after about 24–30 months. Unfortunately, all tubes from these collections were stored at room temperature for part or all of the storage period. Earlier batches at that time had shown no difference in storage life due to temperature. Later observations on several batches from other collections (Table 1: Batches 15 vs. 16 and 17 vs. 18), however, have indicated that deterioration in storage may be more rapid at room temperature than in the refrigerator. More critical experiments are now in progress to compare storage life at −20°, 5°, and 30° C. Although extensive data are available only for 2 field collections, the results so far indicate that spores produced in the field survive dry storage better than do those produced under ordinary greenhouse conditions. It seems likely that any cultural condition (such as temperature, light, humidity, or perhaps host maturity or nutrition) that might affect spore maturity or vigor may influence the storage life of dried spores.

On the assumption that the water content of spores might be an indication of maturity, an examination was made of the storage behavior of lyophil batches from 8 greenhouse collections that had ranged from 8.4 to 16 per cent water (fresh-weight basis) at the time of processing. There was no evidence that storage life was related to water content in these collections.

Two further observations on the use of lyophilized spores are of interest. First, no consistent difference

TABLE 1.—*Summary of processing and storage response data for uredospores of Puccinia coronata and P. graminis f. sp avenae*

Batch	Collection[a]	Days stored before drying[b]	Drying method[c]	Percentage loss in germinability during drying[d]	Storage temperature after drying[e] (°C)	Percentage germination[d] after storage for no. of months indicated in parentheses
P. coronata						
1	I-G	6	F	*34*	C	*41*(0), 36(35)
2	I-G	16	F	*33*	C	*39*(0), 38(35)
3	II-F	1	F	*26*	C	*66*(0), 53(52), 30(65)
4	II-F	34	F	33	C	53(0), 45(51), 35(63)
5	II-F	34	NF	2	C	73(0), 71(32), 51(51)
6	II-F	104	NF	*15*	C	*51*(0), 51(48), 46(62)
7	III-G	0	NF	*39*	C	*55*(0), 46(26)
8	V-G	0	F	34	C	57(0), 50(24)
9	VI-F	8	NF	15	C	75(0), 70(20), 27(40), 37(52)
10	VI-F	8	F		C	53(0), 59(20), 40(39), 33(52)
11	VI-F	12	NF(L)		C	73(0), 51(41), 39(51)
12	VI-F	12	F(L)	40	C	53(0), 58(41)
13	VI-F	16	NF		C	57(0), 58(41), 34(51)
14	VI-F	17	NF(L)		C	40(40), 49(50)
15	VI-F	20	NF(L)		C	64(41), 54(52)
16	VI-F	33	NF(L)		RT	1-2(40)
17	VI-F	150	NF		RT	70(0), 66(17), 10(35), 17(48)
18	VI-F	150	NF		C	70(0), 65(35), 52(39), 22(48)
19	VII-G	0	NF	21	RT-C	69(0), 66(14), 26(32)
20	VIII-G	0	NF	22	RT-C	71(0), 83(14), 30(32)
21	IX-G	0	NF	21	RT-C	66(0), 74(14), 46(32)
22	X-G	0	NF	10	RT-C	75(0), 67(12), 8(31)
23	XI-G	7	NF	8	RT	*76*(0), 22(23), 0-1(35)
24	XII-G	6	NF	37	RT	*49*(0), 17(23), 1(35)
25	XIII-G	7	NF	20	RT-C	*64*(0), 7(23), 5(34)
26	XIV-G	2	NF	15	RT-C	*72*(0), 7(23), 14(34)
P. graminis f. sp. *avenae*						
27	II-G	0	F	50	C	44(0), 50, (43)
28	III-G	30	NF	10	C	67(0), 68(32), 13(42)
29	IV-G	0	NF	25	RT-C	52(0), 28(23), 17(34)
30	V-G	0	NF	25	RT-C	35(0), 45(23), 32(34)

[a] Collection number indicated by Roman numerals; greenhouse and field collections by G and F, respectively; racial composition for *P. coronata:* I-III, 45 and 57; V, 88; VI, 45, 57, and 100; VII, 57; VIII, 88; IX, 98; X, 101; XI-XIV, 57, 88, 98, 101; racial composition for *P. graminis* f. sp. *avenae:* II, mixed; III, 7 and 8; IV, 8; V, 7A.

[b] Stored at about 5°C and 25–50 per cent relative humidity.

[c] F indicates that spores were frozen before lyophilizing and NF that they were not. Small samples were used unless indicated by (L).

[d] A difference in percentage germination of about 12 is significant at the 5 per cent level. Figures in italics were calculated from extrapolated longevity curves.

[e] RT indicates room temperature (20°-35°C); C indicates 2°–5°C; RT-C indicates room temperature until fall of 1953 and 2°–5°C afterward.

in storage life has occurred between the large and small samples of spores during 50 months' storage (Table 1: Batches 13 vs. 14). Second, open lyophil tubes have been stored for as long as 2 weeks in small desiccators at 2°–5°C without change in viability of the spores. Thus spores can be handled in sufficient quantity for any ordinary inoculation work, and a single opened tube may be used more than once.

Experience in spore preservation with other species of *Puccinia* has been more limited. The survival during processing and storage and the assay behavior of spores from 4 greenhouse collections of *P. graminis* Pers. f. sp. *avenae* Eriks. & E. Henn. were quite similar to those of *P. coronata* (Table 1). Single batches from 3 greenhouse collections of *P. graminis* f. sp. *tritici* in 2 cases survived as well as did *P. coronata;* in the third, germination was nil after 30 months. Lyophil batches from 2 field collections of *P. sorghi* Schw. have declined significantly in germination after 28 months' storage.

Time and extent of drying.—Although most of our preservation experience has been with drying times of 2–3 hours, there is evidence that much shorter periods may be sufficient for small samples of spores and possibly even superior. This is illustrated by the data in Table 2; germination of 10-mg samples dried for 15 minutes was equal to or greater than that of those dried 150 minutes.

No similar study of the relationship between drying time and longevity has been made with larger samples of spores, but drying rate curves (method NF) indicated that large samples required somewhat longer times to reach constant weight than did small samples. For example, a 25-mg sample of spores lost 87 per cent of removable water in 10 minutes and reached constant weight after about 50 minutes, whereas a 400-mg sample lost only 54 per cent in 10 minutes and

TABLE 2.—*Germination after storage for various periods at 2 temperatures of uredospores of Puccinia coronata[a] that were lyophilized for various periods*

Storage time (months)	Storage temperature[b]	Percentage germination[c] of batches lyophilized for the indicated no. of minutes		
		15	60	150
0		74	68	75
7	RT	77	74	76
16	RT	71	66[d]	70[d]
34	RT	6	10	6
34	C	71	65	41
39	C	57	52	27
48	C	20	22	23

[a] Batches 17 and 18 of Collection VI (Table 1) lyophilized by Method NF.
[b] RT indicates room temperature (20°–35°C) and C indicates 2°–5°C.
[c] A difference in percentage germination of about 12 is significant at the 5 per cent level.
[d] Required 48-hour rehydration. Others were rehydrated for 24 hours.

reached constant weight after about 90 minutes. The rate of drying by method F varied with the temperature and was much slower than that by method NF (Fig. 1). This may possibly explain the somewhat better survival during drying by the latter method.

The water content of lyophil batches from several collections of uredospores was determined by oven drying at 100°C. These values on a fresh-weight basis ranged from 2–3 per cent for *P. coronata* and from 3–4 per cent for *P. graminis* f. sp. *avenae*. These were all from greenhouse collections that had been stored for various periods in cotton-plugged tubes in the refrigerator, so they may have lost some water before lyophilization. Water content of the large-tube samples of spores of *P. coronata* of 8 lyophil batches from collection VI varied from 1.3 to 2.4 per cent on a fresh-weight basis. There was no evidence that this variation affected the storage life.

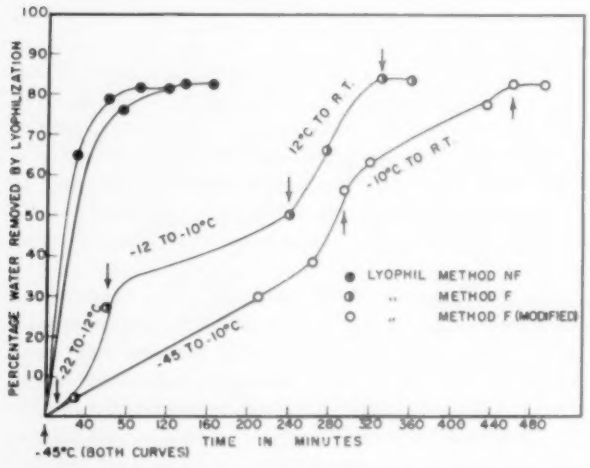


FIG. 1. Drying curves for 400-mg samples of uredospores of *Puccinia coronata* during processing by Method NF (spores not frozen) and by Method F (spores frozen).

TABLE 3.—*Germination and water uptake of lyophilized uredospores of Puccinia coronata[a] that were rehydrated for different periods of time*

Rehydration Hours	Germination[b] %	Water content[c] %
0	0-1	3.4
12	37	34.6
24	45	41.7
36	52	49.1
48	64[d]	52.1

[a] Batch 22 of Collection X (Table 1). Spores were rehydrated after storage for 1 year.
[b] On basis of oven-dry weight.
[c] A difference in percentage germination of about 12 is significant at the 5 per cent level.
[d] No further change occurred with rehydration periods up to 110 hours.

Rehydration.—Extensive experience in assaying lyophilized spores over the past 5 years has amply confirmed the earlier report (12) that dried spores usually require a rehydration treatment to give maximum germination on gelatin or on agar. In nearly all cases, rehydration increased germination several fold; in extreme cases, this was from less than 1 to more than 50 per cent germination. The rehydration time required for maximum germination varied somewhat among spore batches and with conditions of rehydration. When rehydration was in lyophil tubes lightly plugged with cotton in a moist chamber, 24 hours was usually sufficient; however, some batches stored at room temperature required about 48 hours to reach maximum water content and germinability (Table 3). In the 1954 and 1955 assays, spores were rehydrated in thin layers in small open dishes. Comparison of 0-, 12-, 24-, and 48-hour rehydration times with 75 batches showed that rehydration was necessary in 90 per cent of the trials for maximum germination. A time of 12 hours was generally sufficient, and longer than 24 hours was not beneficial in any case. There may be some danger in very long rehydration periods, since some spore lots become too sticky; this makes good distribution of the spores on the plates difficult. A short period of exposure outside the desiccator before inoculation helps to correct this.

Infectivity.—Although maintenance of viability was tested mainly by spore germination, a number of lyophilized samples of spores of *P. coronata* and of *P. graminis* f. sp. *avenae* were tested for infectivity (Table 4). Samples processed by method F always gave better infection if rehydrated before inoculation than if not rehydrated, whereas those processed by method NF did not, except for a sample (PC VII, Batch 19) stored for over a year at room temperature. Other evidence indicates that rehydration may be beneficial for NF samples stored for long periods.

Samples of lyophilized spores of *P. graminis* Pers. f. sp. *secalis* Eriks. & E. Henn., of *P. rubigo-vera* (DC.) Wint. f. sp. *secalis* (Eriks.) Carl., of *P. graminis* f. sp. *tritici*, and of *P. rubigo-vera* (DC.) Wint. f. sp. *tritici* (Eriks. & E. Henn.) Carl. prepared in this laboratory also have been used successfully in green-

TABLE 4.—*Infectivity to oats and germinability of nonlyophilized and lyophilized uredospores of Puccinia coronata (PC) and of P. graminis f. sp. avenae (PGA) with and without rehydration*

Collection and batch [a]	Duration of storage [b] (months)	Not rehydrated: Percentage germination	Not rehydrated: Infection rating [c]	Rehydrated: Percentage germination	Rehydrated: Infection rating [c]
		(Nonlyophilized spores)			
PC I	11	0-1	0	0-1	0
PC II	10	50	+++		
PC II	20	10	++	13	++
PC II	22	0-1	0	0-1	0
PGA II	13	0-1	0	0-1	0
		(Lyophilized spores)			
PC I, 1	24	0-1	+	40	+++
PC II, A	28	0-1	+	53	+++
PC II, B	30	0-1	+	50	+++
PC II, 6	28	3	+++	49	+++
PGA II, 27	22			45	++
PC VII, 19	13	0-1	+	65	+++

[a] Collection and batches as listed in Table 1 except PC II, A (which was similar to PC II, 4) and PC II, B (which was similar to PC II, 5 but was dried by Method F).

[b] Nonlyophilized samples were stored at about 5°C and 25–50 per cent relative humidity. Lyophilized samples were stored at 2°–5°C except that PC VII, 25 was stored at room temperature (20°–35°C).

[c] Infection ratings: +++ = over 25 pustules/cm²; ++ = 5–25/cm²; + = less than 5/cm²; 0 = no pustules.

house inoculations (the first 2 by R. W. Earhart, the last 2 by W. C. Haskett).

A quantitative comparison of the infectivity of lyophilized and nonlyophilized spores is shown in Table 5. About 75 per cent of each were viable. Measured amounts of each in water suspension were brushed on leaves. The results showed no difference in infectivity of lyophilized and nonlyophilized spores on the basis of pustule counts, and the extent of infection was roughly proportional to the amount of inoculum.

A comparison of various inoculation methods was made in tests in which equal weights of spores were applied to each pot. With leaves debloomed by hand, brush and atomizer, application of spore suspension gave no statistically significant difference in pustule counts. Spores applied in talc suspension or in water suspension following deblooming by Dreft (a household detergent manufactured by Procter and Gamble) solution gave significantly lower counts. Lyophilized spores also were effective when inoculation was by hypodermic injection near the growing point, both in the greenhouse and in the field. The results indicate that any of several commonly used inoculation methods are satisfactory for lyophilized spores.

Four lyophilized pure race collections of *P. coronata*, which had been in storage for a year, were tested

TABLE 5.—*Comparative infection of oats by nonlyophilized and lyophilized uredospores [a] of Puccinia coronata of equal germinability*

Spores applied per pot of 6 seedlings (mg)	Pustules per cm² of leaf [b]: Nonlyophilized	Lyophilized
2	125	133
1	65	58
0.5	38	30
0.25	15	18

[a] Lyophilized spores were those of Collection VII, Batch 19 (Table 1) stored for 10 months; nonlyophilized spores were a recently harvested collection of similar racial composition.

[b] Average of 10 leaf sections taken at random.

for stability of infection type on selected host differentials. No change in mean infection type after drying and storage was detected.

Barratt-Tatum technique.—The success of the NF method (with the desiccant water trap) and the possible need for a simple apparatus for small-scale use led to a test of the Barratt-Tatum technique (2). In this experiment, 5 cm of desiccant was placed between cotton plugs and about 8 cm above a 5- to 10-mg spore sample in a 25-cm lyophil tube. Such tubes were connected through a 4-branch manifold directly to the pump. After 5 minutes' evacuation to a pressure of about 50 μ of Hg, the tubes were sealed off above the desiccant. One series was sealed off again below the desiccant after 3 hours, and the other was stored with desiccant remaining to determine whether the second sealing could be avoided.

Table 6 summarizes the results of this experiment after about a year's storage at room temperature. With the desiccant removed before storage, only P_2O_5 failed to give as good storage as did the regular NF method. When the desiccant was left in the tubes, all 4 treatments resulted in loss of viability. The drop was greatest with P_2O_5 and $MgClO_4$, which are the most powerful desiccants used; this suggests that removal of water to a very low level may reduce storage life. A similar set of treatments in which a water-pump vacuum of a pressure of 12 mm of Hg was used

TABLE 6.—*Germination of uredospores of Puccinia coronata [a] that were dried by a modification of the Barratt-Tatum technique*

Desiccant	Storage (days)	Percentage germination: Desiccant removed	Percentage germination: Desiccant not removed
P_2O_5	9	73	55
	347	54	16
$MgClO_4$	9	73	62
	347	64	15
Silica gel	9	75	72
	347	70	53
Drierite [b]	9	76	74
	347	65	51

[a] Collection X (Table 1).

[b] Anhydrous $CaSO_4$ (W. A. Hammond Co).

resulted in less than 10 per cent viable spores after only 80 days. Apparently, either the drying was not sufficiently rapid or complete or the presence of residual oxygen during storage was deleterious. Although the Barratt-Tatum technique has not yet been tested for long enough periods, the results so far indicate that this method may be equivalent to the regular technique provided a suitable desiccant is used and the desiccant is removed before storage. Although less special equipment is required, this technique is slower than the NF method because of the necessity of loading individual tubes and because of the 2 sealing operations.

Discussion.—The essential features of this preservation method appear to be the reduction of spore water content by high-vacuum drying to 2–3 per cent (on oven-dry basis) and storage in the absence of oxygen and water vapor. The usual quick freezing before evacuation has not been found necessary with Puccinia uredospores. This raises the question of whether the water actually is removed by ice sublimation, i.e. whether, in fact, the process actually is lyophilization. No conclusive experimental answer was obtained, but the rapid evacuation of the system and the large evaporation surface make it quite likely that rapid freezing occurred. Another difference from the usual lyophil process was the omission of aqueous hygroscopic suspending media. This reduces the drying time and leaves the dried spores in a convenient condition for assay and inoculation. The necessity for exclusion of oxygen and water vapor during storage was first indicated by the rapid deterioration of spores in several lyophil tubes that developed small cracks after sealing. This was confirmed by the observation that spores stored in wet air, in wet nitrogen, or in dry air deteriorated much more rapidly than did those stored in dry nitrogen or vacuum.

The rate and extent of drying are 2 other factors of possible importance in the method. The evidence bearing on rate in the present study is from comparison of results with large and small samples of spores and from comparisons of methods F and NF. Times for maximum water removal in these comparisons ranged from about 20 to 480 minutes. With the exception of somewhat smaller viability losses during the more rapid drying in method NF, however, there was no clear evidence that storage life was affected by the drying rate. Furthermore, the small variations in extent of drying observed in several experiments with the regular methods did not appear to affect storage life. The only indication that excessive drying might be harmful was the poor survival when powerful desiccants were used in the Barratt-Tatum technique. Although the influence of rate and extent of drying on storage life has not so far proved critical, further examination is desirable, and such experiments are in progress.

Another critical feature of the present method is the necessity for slow rehydration of the dried spores. In the dry state, the protoplast is shrunken and the spore wall partially collapsed. Perhaps reimbibition is too rapid and stresses that damage the protoplast are created when the spores are in contact with liquid water (11).

In an evaluation of the present status of the drying method for uredospore preservation from a practical standpoint, it is of interest to compare storage life by this method with that by refrigeration, which has been the common method. Such a comparison can be made for a number of collections used in the present study on the basis of the storage time required for the germination to decrease to about one-quarter of that at the beginning of storage. For cold-storage spores, these times have ranged from a minimum of 2 to a maximum of over 12 months. The ratios of such times for lyophilized batches to those for the corresponding cold-storage spores have ranged from about 4 for some of the greenhouse collections to over 12 for other batches. Since the lowest ratios involved room-temperature storage of dried spores, it is likely that the minimum ratio is greater than 4; in other words, lyophilized spores can be expected to last more than 4 times as long as nonlyophilized spores in cold storage.

It has become increasingly clear during the last 3 years of this study that spore collections vary considerably in their potential storage life in the dry state as well as in cold storage. This appears to be largely a property of the spores themselves rather than a function of the drying method; it would seem to depend on the conditions under which the spores are produced. Aside from the apparent difference between field- and greenhouse-produced spores, data from the collections available in the present work shed no light on the cultural factors that influence storage life. To answer this question, it would be necessary to examine the storage behavior of spores grown under controlled conditions. This may also be necessary to determine whether there is any difference among races in this respect.

Because of the difficulty of quantitative measurements of infectivity, this study has been based largely on germination assays. In our experience, however, the latter has been a fair measure of inoculum potential, and lyophilized spores gave good infection when properly handled. Perhaps the most critical question at present is whether there is any danger of change in pathogenicity during processing or storage. The limited tests with the present material have revealed none, but more evidence on this question is needed. No reports of such variations in lyophilized fungi have been found (cf. 3), but there is some evidence of loss or change in certain physiological characteristics by yeast and bacteria (1, 14).

Department of Botany and Plant Pathology
Iowa State College
Ames, Iowa

LITERATURE CITED

1. Atkins, L., W. Moses, and P. H. Gray. 1949. The preservation of yeast cultures by lyophilization. J. Bacteriol. **57**: 575-578.

2. Barratt, R. W., and E. L. Tatum. 1950. A simplified method for lyophilizing microorganisms. Science **112**: 122-123.
3. Fennell, D. I., K. B. Raper, and M. H. Flickinger. 1950. Further investigations on the preservation of mold cultures. Mycologia **42**: 135-147.
4. Flosdorf, E. W. 1949. Freeze-drying. Reinhold Publ. Corp. New York. 280 p.
5. Forbes, I. L. 1939. Factors affecting the development of Puccinia coronata in Louisiana. Phytopathology **29**: 659-684.
6. McCallan, S. E. A., and F. Wilcoxon. 1932. The precision of spore germination tests. Contribs. Boyce Thompson Inst. **4**: 233-243.
7. Maneval, W. E. 1924. The viability of uredospores. Phytopathology **14**: 403-407.
8. Murphy, H. C., and M. N. Levine. 1936. A race of crown rust to which the Victoria oat variety is susceptible. Phytopathology **26**: 1087-1089.
9. Raper, K. B., and D. F. Alexander. 1945. Preservation of molds by the lyophil process. Mycologia **37**: 499-525.
10. Rosen, H. R., and L. M. Weetman. 1940. Longevity of uredospores of crown rust of oats. Arkansas Agr. Expt. Sta. Bull. 391, 20 p.
11. Rowell, J. B. 1956. Rehydration injury of dried uredospores of Puccinia graminis var. tritici. (Abs.) Phytopathology **46**: 25.
12. Sharp, E. L., and F. G. Smith. 1952. Preservation of Puccinia uredospores by lyophilization. Phytopathology. **42**: 263-264.
13. Snedecor, G. W. 1946. Statistical methods. Ed. 4. Iowa State College Press. Ames, Iowa. 485 p.
14. Wolf, H. L. 1952. Experiments with an apparatus for lyophile desiccation of microorganisms. Antonie van Leeuwenhoek J. Microbiol. Serol. **18**: 113-124.

INHIBITION OF FUSARIUM OXYSPORUM F. LYCOPERSICI BY A SPECIES OF MICROMONOSPORA ISOLATED FROM TOMATO[1]

Glenn E. Smith[2]

SUMMARY

An orange-colored organism was isolated from a tissue section of an apparently healthy Bonny Best tomato plant previously inoculated with *Fusarium oxysporum* f. *lycopersici*. It was tentatively identified as a species of the genus *Micromonospora*.

Micromonospora sp. had an inhibiting effect on *F. oxysporum* f. *lycopersici* when the 2 organisms grew near each other on potato-dextrose agar in Petri dishes. *F. oxysporum* f. *lycopersici* did not grow on agar in which *Micromonospora* sp. had grown for 1 day or longer.

A 50 per cent concentration of a crude filtrate from broth cultures of the *Micromonospora* sp., when added to agar, inhibited *F. oxysporum* f. *lycopersici*, and pigment formation was intensified. *Micromonospora* sp. was reisolated from 4 of 20 tomato plants inoculated with the organism alone; *Micromonospora* sp. induced no macroscopic symptoms in tomato.

Symptoms of Fusarium wilt were inhibited in tomato plants treated with Micromonospora cultures before they were planted in Fusarium-infested soil or inoculated by swirling the roots in broth cultures of *F. oxysporum* f. *lycopersici*. Plants were treated by immersing their roots in broth cultures of *Micromonospora* sp. for from 1 hour to 2 days. Symptom retardation was most striking when roots of tomato plants were in various concentrations of Micromonospora filtrate for a period of 4 days before inoculation with *F. oxysporum* f. *lycopersici*.

In a class experiment, 15 Bonny Best tomato plants were inoculated with race 1 of *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans. (hereafter referred to as Fusarium). Twenty days later, 14 of the plants had typical wilt symptoms whereas 1 plant appeared to be as healthy as the noninoculated check plants. The stem, above the crown, was cut diagonally and examined, but the vascular system was not discolored. The cut stem was placed in water for a few days until roots developed and then was transplanted to steamed soil in a clay pot. Tissue cultures were made from the petioles of lower leaves to determine whether or not Fusarium was present. An orange-colored organism grew from 1 section whereas 15 of 16 other tissue sections were sterile. The organism was tentatively identified as a member of the genus *Micromonospora*. The discovery of the *Micromonospora* sp. in this 1 plant instigated investigations of the inhibitory properties of *Micromonospora* sp. on Fusarium *in vitro* and *in vivo*.

Experimental methods and results.—*Inhibition of Fusarium by Micromonospora sp. in vitro.*—A small volume of a 5-day-old broth culture of *Micromonospora* sp. was streaked from a wire loop onto the surface of potato-dextrose agar in Petri dishes, and a small colony of Fusarium was placed about 6 cm from the streak. At the end of 8 days, the Fusarium was inhibited (Fig. 1, A).

When dishes of agar in which *Micromonospora* sp. had been growing for 1 hour, 1 day, 2 days, and 4 days were cross streaked with Fusarium, the inhibition of

[1] Accepted for publication March 2, 1957.
Paper No. 596, Department of Botany and Plant Pathology, the Ohio State University. Portion of a thesis submitted to the Graduate School of The Ohio State University in partial fulfillment of the requirements for the Master of Science Degree.
[2] The author wishes to thank Dr. C. C. Allison, his adviser, for his helpful suggestions and criticisms during the course of this work.

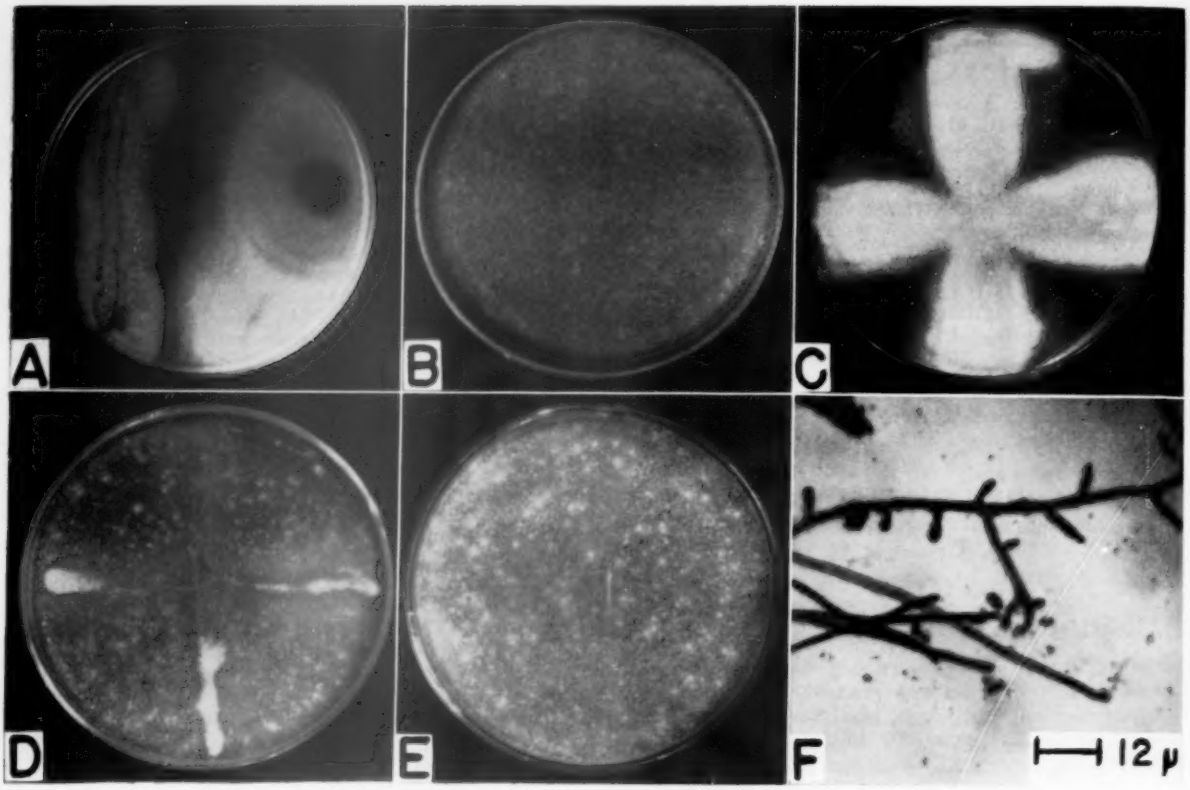


FIG. 1. A) Inhibition of *Fusarium oxysporum* f. *lycopersici* (right) by *Micromonospora* sp. (left), when margins of colonies were in close proximity (8-day-old colonies). B) Agar plate 8 days after it was seeded with *Micromonospora* sp. C) Agar plate 8 days after it was cross streaked with *F. oxysporum* f. *lycopersici*. D) Agar plate seeded with *Micromonospora* sp., cross streaked 1 hour later with *F. oxysporum* f. *lycopersici*, then photographed 8 days later. E) Agar plate seeded with *Micromonospora* sp., cross streaked 1 day later with *F. oxysporum* f. *lycoporsici*, then photographed 7 days later. F) Photomicrograph of mycelium of *Micromonospora* sp. Note single spores on short lateral branches.

Fusarium was striking. The dishes were prepared by adding standard amounts of Micromonospora spores and mycelium to warm agar. Dishes containing sterile agar were streaked similarly at the same intervals. No visible growth of Fusarium was observed when *Micromonospora* sp. had grown 1 day or longer before Fusarium was added. Representative dishes were photographed (Fig. 1, B,C,D,E).

A crude filtrate of a 45-day-old Micromonospora culture in potato-dextrose broth[3] was sterilized by passage through a Seitz filter. This filtrate and water were added to warm double-strength potato-dextrose agar in amounts to make single-strength potato-dextrose agar containing 10 and 50 per cent filtrate. Eight Petri dishes at each concentration and 8 dishes containing potato-dextrose agar only were poured and autoclaved. A small Fusarium colony was transferred to the center of each dish. These were incubated at room temperature for 8 days and the diameter of the colonies measured daily.

[3] Per liter: Extract from 50 g of sliced, peeled potatoes autoclaved 20 minutes in 500 distilled water; dextrose 5 g.

Smaller colonies grew on the agar containing 50 per cent than on agar containing 10 per cent filtrate or on potato-dextrose agar alone. According to the statistical analysis of the group data for the colonies on potato-dextrose agar and those for colonies on the agar containing the 50 per cent filtrate concentration, the mean difference between the colony diameters was significant at the 1 per cent level. Age of culture was disregarded in this analysis.

The Fusarium colonies on the filtrate-containing media were more pigmented than normal; the intensity of the purple pigmentation was roughly proportional to the amount of filtrate in the medium.

Inhibition in vivo.—Thirty Bonny Best tomato plants, at the 4-leaf stage, were removed from flats and their roots washed free of soil. Each of 15 plants was placed in a 125-ml Erlenmeyer flask containing a 5-day-old Micromonospora culture growing in potato-dextrose broth; each of the remaining plants was placed in a flask containing water. In both cases, enough water was added to cover the root systems of the plants. One hour, 1 day, and 2 days later, 5 treated and 5 untreated plants were transplanted to Fusarium-infested soil in flats; treated and untreated plants were alter-

nated in each row. At the end of 30 days, each plant was classified according to a slight modification of Wellman's (6) rating system; ratings were on a scale of 0 to 10, with 0 indicating no symptoms and 10 indicating very severe symptoms. Treated plants had a mean Wellman rating of 1.3 compared to one of 4.5 for the nontreated ones.

Because of the time element and the uncertainty of infection by Fusarium of tomato roots growing in Fusarium-infested soil, an inoculation method similar to that described by Weindling and Armstrong (5) was used when the experiment was repeated; the roots of each plant were swirled in a 5-day-old broth culture of Fusarium diluted with water and then left in the culture for 10 minutes. Fusarium-inoculated plants (previously treated or not treated with Micromonospora) were transplanted to flats of steamed soil. Fourteen days after inoculation, each plant was classified according to Wellman's rating. The mean rating for Micromonospora-treated plants was 1.9, whereas that for those not treated with Micromonospora was 6.2.

In another experiment, a few roots of each plant were cut before inoculation with Fusarium. The time intervals and methods were the same as those used previously, with the exception that the untreated plants were placed in potato-dextrose broth instead of water. Also, the total number of plants used was 60 (Table 1). According to the statistical analysis of the group data for tomato plants treated with Micromonospora cultures and of those for tomato plants treated with potato-dextrose broth, the difference between the mean Wellman ratings was significant at the 1 per cent level. Within each time interval, the difference between the mean Wellman ratings for plants treated with potato-dextrose broth and that for those treated with Micromonospora culture was not significant. With plants treated only with broth, the differences between the average Wellman ratings for each time interval were not significant.

TABLE 1.—*Fusarium wilt ratings of tomato plants whose roots had been placed in 5-day-old Micromonospora broth cultures or in potato-dextrose broth for various periods of time before inoculation with Fusarium oxysporum* f. *lycopersici*

Treatment[a]	Time	Wellman ratings[b] Range	Average
Roots in PDB	1 Hour	4–10	8.4
	1 Day	4–10	6.3
	2 Days	4– 7	5.1
			Mean[c] 6.6
Roots in MC	1 Hour	2– 9	4.2
	1 Day	0– 6	3.0
	2 Days	1– 4	2.2
			Mean[c] 3.1

[a] PDB = potato-dextrose broth; MC = Micromonospora culture.
[b] Based on a scale of 0 to 10, with 0 indicating no symptoms and 10 very severe symptoms.
[c] LSD of means is 2.14 at the 1 per cent level.

A modification of the method of Gopalkrishnan and Jump (1) was used in experiments on the effect of the introduction of Micromonospora filtrate into tomato plants on symptoms of Fusarium wilt. Forty seedling tomato plants, at the 4-leaf stage, were removed from flats, their roots washed free of soil, and each of 10 plants placed in a flask containing enough Knop's solution (3) to cover the roots. Other groups of 10 plants each were placed in individual flasks of Knop's solution containing 1, 10, or 50 per cent Micromonospora filtrate. The filtrate was sterilized by passage through a Seitz filter, and double-strength Knop's solution and water were used for dilution so that each solution would be equivalent to single-strength Knop's solution. After 4 days, the treated plants were removed, a few roots of each plant were cut, and the remaining roots of each plant were swirled in a 5-day-old broth culture of Fusarium and left in the culture for 10 minutes. The plants were placed in steamed quartz sand in clay pots and watered as needed with Knop's solution. At the end of 14 days, each plant was classified according to Wellman's rating. According to these data (Table 2), all concentrations of Micromonospora filtrate used reduced Fusarium wilt ratings.

Platings were made of all plants inoculated with Fusarium. Root and stem sections were submerged in 0.001 per cent $HgCl_2$ for 30 seconds, then in 2.6 per cent sodium hypochlorite for 1 minute. They were transferred to potato-dextrose agar in Petri dishes and incubated at room temperature for 6 days. Fusarium was recovered from all Fusarium-inoculated tomato plants. *Micromonospora* sp. was recovered once from tissue sections of tomato plants treated with Micromonospora cultures before inoculation with Fusarium.

Effect of Micromonospora alone on tomato plants.—Twenty seedling tomato plants, at the 4-leaf stage, were removed from flats and their roots washed free of soil. Each plant was placed in a flask containing a 5-day-old Micromonospora culture growing in potato-dextrose broth. One hour later and 1 day later, 10 treated plants were transplanted to steamed soil. At the end of 14 days, the Wellman rating for all plants was zero and no macroscopic symptoms were observed. *Micromonospora* sp. was reisolated from 4 of 20 Micromonospora-inoculated plants. Growth of Micromono-

TABLE 2.—*Fusarium wilt ratings of tomato plants whose roots were immersed for 4 days in Knop's solution containing various concentrations of Micromonospora filtrate prior to inoculation with Fusarium oxysporum* f. *lycopersici*

Percentage concentration of filtrate in Knop's solution	Wellman rating[a] Range	Average[b]
0	6–9	7.1
1	4–6	4.8
10	1–3	1.2
50	1–4	1.7

[a] Based on a scale of 0 to 10, with 0 indicating no symptoms and 10 very severe symptoms.
[b] LSD of averages is 1.14 at the 1 per cent level.

spora from tissue sections was slow—9 to 15 days at room temperature.

IDENTIFICATION OF MICROMONOSPORA SPECIES.—The following characteristics tentatively placed the orange-colored organism in the genus *Micromonospora* of the order Actinomycetales. The mycelium is nonseptate and well developed, with a mean diameter of 1 μ on dextrose-asparagine agar and 1.8 μ in potato-dextrose broth. Single spores are attached to short lateral branches (Fig. 1,F). Abundant spores are produced when the organism grows on potato-dextrose agar. It is pale orange when it grows on dextrose-asparagine agar. It is Gram positive and diastatic but not acid fast. Metabolites of Micromonospora are inhibitory to Fusarium *in vitro* and reduce Fusarium wilt symptoms in tomato.

DISCUSSION.—The inhibition of Fusarium is apparently the first report of the inhibition of a fungus by a species of *Micromonospora* or by its filtrate, although there have been reports of the inhibition of bacteria by such species. Welch (7) observed that a filtrate from a Micromonospora culture inhibited the growth of certain bacteria, and Waksman (4) isolated from a species of *Micromonospora* an antibiotic (micromonosporin) that was active against various Gram-positive bacteria.

The effect of the Micromonospora filtrate on pigment formation in Fusarium is similar to the phenomenon observed by Grossbard (2), who noticed intensification of color in Fusarium growing on agar near paper discs impregnated with crude unidentified antibiotics produced by Actinomycetes. She suggested that this phenomenon was frequently associated with antibiotics.

Micromonospora sp. may enter roots and grow into the vascular system of the stem, or spores may enter roots and be transported in the vascular system. The relationship of *Micromonospora* sp. to tomato host tissue and the effect on Fusarium has not been established. One explanation for the low recovery of *Micromonospora* sp. from tomato plants treated with Micromonospora cultures and then inoculated with Fusarium lies in the slow growth of *Micromonospora* sp. from tissue sections. Fusarium is a rapid grower in comparison and quickly overgrows agar near tissue sections.

Reduction of Fusarium wilt symptoms by Micromonospora cultures and by Micromonospora filtrate possibly involves systemic action of an antibiotic. When tomato plants were placed in various concentrations of filtrate, the lower disease ratings were associated with the higher filtrate concentrations. In the 5-day-old Micromonospora cultures used for treatment, perhaps sufficient antibiotic was present to reduce wilt symptoms of the treated plants. Evidence that antibiotics pass through roots was obtained by Gopalkrishnan and Jump (1), who found that tomato plants treated with thiolutin (an antibiotic obtained from *Streptomyces albus*) and then inoculated with Fusarium remained free from Fusarium wilt symptoms, although stunting occurred at higher concentrations and Fusarium was reisolated from plants treated at lower concentrations.

Some possibilities exist for the control of Fusarium wilt of tomato by the use of *Micromonospora* sp. in commercial greenhouses and fields. One of these is soil infestation with Micromonospora spores and mycelium at the time of transplanting. Another is the dipping of roots into an antibiotic (if the purified active chemical of this particular Micromonospora filtrate can be isolated) at the time of transplanting.

Specific identification of the organism remains tentative, but it apparently is not the same as any described species.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY
THE OHIO STATE UNIVERSITY
COLUMBUS 10, OHIO

LITERATURE CITED

1. GOPALKRISHNAN, K. S., AND J. A. JUMP. 1952. The antibiotic activity of thiolutin in the chemotherapy of the Fusarium wilt of tomato. Phytopathology **42**: 338-39.
2. GROSSBARD, ERVA. 1954. Effect of metabolites of Actinomycetes on pigmentation and spore formation in phytopathogens. Phytopathology **44**: 110-11.
3. MCLEAN, R. C., AND W.R.I. COOK. 1941. Plant science formulae. Macmillan and Co, London. 203 p.
4. WAKSMAN, S. A., W. B. GEIGER, AND ELIZABETH BUGIE. 1947. Micromonosporin, an antibiotic substance from a little-known group of microorganisms. J. Bacteriol. **53**: 355-57.
5. WEINDLING, R., AND G. M. ARMSTRONG. 1939. A water-culture infection method used in the study of Fusarium. Phytopathology **29**: 23.
6. WELLMAN, F. L. 1939. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopathology **29**: 945-55.
7. WELCH, M. 1952. Bacteriostatic and bacteriolytic properties of Actinomycetes. J. Bacteriol. **44**: 571-88.

CONTROL OF CITRUS FRUIT RUSSET IN FLORIDA WITH ZINEB [1]

Fran. E. Fisher

SUMMARY

Russet of citrus fruits, long combatted by sulfur on the theory that it was caused by the rust mite *Phyllocoptruta oleivora* (Ashm.), was controlled effectively in Florida by a single application of zineb during July or early August. Although zineb depressed populations of rust mites, there is some evidence to suggest that russet may be caused not by mites but by a fungus, possibly *Cladosporium brunneo-atrum* McAlp.

Russet of fruits has long been a major problem in the growing of citrus in Florida. Affected fruit are seriously blemished, often smaller in size than normal, and of low grade in the packinghouse. For more than half a century, russet has generally been assumed to be caused by the rust mite, *Phyllocoptruta oleivora* (Ashm.), and control of this mite with sulfur has been recommended since 1885 (7). This and other maladies attributed to the rust mite has led to the use of some 50,000 tons of sulfur annually in Florida. The purpose of this paper is to report control of russet through the use of fungicides, particularly zineb.

DEFINITION OF TERMS AND OF THE PROBLEM.—As referred to in this paper, russet is an external blemish of citrus fruits that appears after fruits are almost full size. Injured areas range in color from bronze through shades of brown to black. With some forms of injury, affected areas have a smooth surface that will take a high degree of polish in the packinghouse. Various types of russet are given colloquial names. A slight russeting of oranges is called "golden." In the most severe form, "black russet," the surface layers tend to become thickened and ultimately develop a "mud-cake" appearance resembling mud-cake melanose (due to *Diaporthe citri* Fawc.). Another form, also similar to melanose, is referred to as "tear stain." Miscellaneous patterns, generally brown in color, are known simply as "russet." Fruit unaffected by the disease is referred to as "bright." Figure 1 shows various types and degrees of russet on Ruby Red grapefruit.

Russet not only lowers the grade of fruit, but also prevents the development of normal sizes. According to Yothers and Mason (16), diseased fruits are about 12½ per cent smaller than those unaffected; many are unmarketable. They dehydrate and decay more rapidly than do healthy fruits. The longer that affected fruit remains on the tree, the larger the percentage of loss to the grower.

During the 1870's, citrus growers in Florida believed russet to be due either to fungi or to adverse soil conditions. During this period, the rust mite and its association with the disease was discovered by J. K. Gates (16). The report of a disease bearing strong resemblance to "tear-stain" russet seems not to have been noticed in Florida. This disease, called "false melanose," was shown to be caused by *Cladosporium brunneo-atrum* McAlp. and was described and illustrated in color in Australia in 1899 by McAlpine (8). McAlpine considered this type of injury to be distinctly different from that due to rust mite.

Russet has never been reproduced under controlled conditions. When Ashmead (1) in 1879 described the rust mite, he gave it the specific epithet *oleivora*, believing that the mites ruptured the oil cells which in turn resulted in russeting (or burning) of the rind. All attempts by Yothers and Mason (16) to reproduce russet artificially either by rupturing the oil cells or with rust mites failed. Neither did Winston (15) succeed in confirming Rolfs' (11) claim that "tear-stain" russet was produced by *Colletotrichum gloeosporioides* Penz.

The facts that sulfur controlled rust mites, that russet was either entirely absent or a minor problem when rust mites were not numerous, and that russet apparently was prevented by controlling rust mites with sulfur all supported the belief that russet is in actuality an injury due to rust mites. The intensity of russeting, however, is not always correlated with the number of rust mites.

PROCEDURES.—Tests reported here cover spray trials carried out under grove conditions in 1954, 1955, and 1956. Each commercial grove in which tests were made received the usual sprays in spring and fall months, but during summer months no treatments were applied other than the experimental materials. All treatments were applied with hand spraying equipment so as to wet trees thoroughly, both inside and outside. Because of the waxy nature of citrus, each spray material was supplemented in 1955 and 1956 with a surface-active agent, Sprayspread,[2] at a rate of ½ oz. per 100 gal. with mixtures containing oil and 1 oz. per 100 gal. in others.

Materials used in screening tests[3] in 1954 were pri-

[1] Accepted for publication February 24, 1957.
Florida Agricultural Experiment Stations Journal Series No. 594.

[2] A formulation containing an ester of glycerol mannitan laurate (Niagara Chemical Division, Food Machinery and Chemical Corporation).

[3] The writer wishes to thank the following manufacturers from whom free materials for this study were received: Niagara Chemical Division of Food Machinery & Chemical Corp., Rohm & Haas Co., Stauffer Chemical Co., Chas. Pfizer & Co., E. I. du Pont de Nemours Co., Woudhuysen & Associates, California Spray-Chemical Co., Upjohn Co., Commercial Solvents Corp., and American Cyanamid Co.

FIG. 1. Various types and degrees of russet on Ruby Red grapefruit. Fruit in upper left hand corner illustrates the "tear-stain" type of russet.

marily those being tested for control of greasy spot. Packinghouse records were taken from these tests to learn the effect of the same materials on russet. Materials used in subsequent tests in 1955 and 1956 were selected from those found promising in the original screening tests; the objective was to determine if a single application of any 1 material would give control of both diseases.

Screening tests in 1954 were made in 2 groves with a total of 11 fungicides and 5 antibiotics. Two to 4 applications were made between May 2 and November 17 on plots of various sizes.

In 1955, zineb (zinc ethylenebis[dithiocarbamate]), captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide), ziram (zinc dimethyldithiocarbamate), basic copper sulfate, and silver salicylate were chosen from the above tests for further field trials on Pineapple and Valencia oranges. In addition, oil emulsion, parathion, and wettable sulfur plus lime-sulfur were used, these being materials that are regularly used for the control of citrus insects and mites. Two groves were each divided into 3 series of 4-tree plots encircled by buffer rows and replicated 4 times. One treatment was applied to each plot. The first series received treatments at the end of June, the second in the middle of July, and the third at the end of July, except that Valencias were sprayed early in August.

Materials and combinations used in 1956 were applied to determine not only the lowest dosage rate necessary for control but also the effect of zineb with oil emulsion and with parathion, one or both of which are ordinarily used in summer for the control of scale insects. If a combined spray could be applied at the regular time for the control of both scale and russet, it would mean a large reduction in application expense. A Ruby Red grapefruit grove was divided into 3 series of 4-tree plots replicated 3 times for each spraying date. The first series of treatments was applied on July 2, the second on July 16, and the third on August 3.

A grove of Hamlin orange trees was divided into 3 series of 12-tree plots replicated 3 times for each date of treatment. The first series received treatments on July 2, the second on July 16, and the third on July 30. A Duncan grapefruit grove was divided into 9- and 12-tree plots replicated 4 times and was sprayed on July 17. A tangerine grove with 9-tree plots replicated 4 times was sprayed on July 18. Other varieties on which tests were made but not reported in detail here included Valencia orange, Marsh grapefruit, Temple orange, and Jaffa orange.

RESULTS.—Zineb, captan, silver salicylate, and ziram were selected from the 1954 screening tests as fungicides that showed most promise for the control of both russet and greasy spot. Other materials that decreased russeting were maneb (manganous ethylenebis[dithiocarbamate]), nabam (disodium ethylenebis[dithiocar-

bamate]) plus zinc sulfate, ferbam (ferric dimethyldithiocarbamate), Agri-mycin (15% streptomycin, 1.5% oxytetracycline), cycloheximide (3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide), neomycin sulfate, ascosin, and C-17 (an experimental antibiotic similar to ascosin).

Tests in 1955 showed that in both groves trees sprayed with a single application of zineb between June 29 and August 3 had more bright fruit than did those receiving any of the other 7 treatments. The following records were made on Pineapples treated the end of July and Valencias treated the first part of August. Pineapple oranges sprayed with zineb were 95 per cent bright; Valencias, 90 per cent. Pineapple oranges sprayed with sulfur were 85 per cent bright; Valencias ranged between 20–50 per cent bright fruit. On both varieties, fruits treated with captan were comparable to those treated with sulfur. Bright fruit on non-sprayed controls constituted only 10–20 per cent of the crop.

Packinghouse records for the 1956 crop of Ruby Red grapefruit and Hamlin oranges are given in Table 1. Comparisons between treatments are shown in Figure 2. Packinghouse records of Duncan grapefruit harvested on October 26 showed that a July 17 application of zineb (0.65 lb./100 gal.) resulted in 99 per cent bright fruit whereas the nonsprayed control had 1.5 per cent bright fruit. Tangerines had 99 per cent bright fruit on trees sprayed with oil plus parathion plus zineb (0.65 lb./100 gal.), and those treated with oil-parathion had 75 per cent bright fruit. In general, russet is not as severe on tangerines as on grapefruit and round oranges.

Packinghouse records showed that zineb applied between July 2 and August 3, alone or in combination with oil emulsion plus parathion, controlled russet on Ruby Red grapefruit, Duncan grapefruit, Hamlin oranges, and tangerines. Field examination of later maturing varieties showed similar results. In each grove where the disease was present on nonsprayed controls, zineb was found to control the disease as well as, if not better than, did sulfur on adjoining groves where up to 5 summer sulfur sprays or dusts were applied.

Results consistent with the above-described control of russet have been obtained in approximately 200 acres of commercial citrus sprayed with zineb in 1956. Groves receiving zineb on a commercial basis were located at various points up to 70 miles from the experimental plots.

Control of russet with zineb suggests that the blemish is caused by a fungus. In the laboratory, fungus mycelia were found in the epidermis of diseased fruit. A search was made subsequently among overmature, shrivelled, russeted fruits in the field. Conidiophores and conidia corresponding to the description of *Cladosporium brunneo-atrum* McAlp. were found on some of these fruit. Other fruits that were severely diseased but exhibited no fruiting structures were placed in moisture chambers. Subsequently, conidiophores and conidia resembling those of *C. brunneo-atrum* developed.

Some consideration has been given to the possibility that in the control of russet, zineb functioned not as a fungicide but as a miticide. Accordingly, terminal counts were made of rust mites in sprayed and check-sprayed plots. On November 17, 1956, the rust mite population on zineb-sprayed Duncan grapefruit was 0.93 per cent, whereas on oil-parathion sprayed fruits the rust mite population was 41.3 per cent. Although no reports have been found that zineb acts as an acaracide, this possibility is being investigated further.

Discussion.—Many operations in the growing of Florida citrus are centered around the rust mite. Positive correlations have been established, on the basis of field data, linking the rust mite with 3 different diseases: 1) greasy spot (2, 13), 2) rind pitting (13), and 3) false melanose or tear stain (15). These diseases, however, have been known to occur with severity when rust mite populations were insignificant. It should be mentioned at this point that some workers have maintained that these diseases are caused by agencies other than the rust mite. Thus, Tanaka and Yamada (12) claim that greasy spot is due to the *Cercospora* stage of *Mycosphaerella horii* Hara; Fawcett (3) states that rind pitting is caused by *Colletotrichum gloeosporioides* Penz.; and McAlpine (8) reports false melanose to be caused by *Cladosporium*

Table 1.—*Packing-house records of the percentage of bright (russet-free) fruits from citrus groves near Lake Alfred sprayed at various dates with zineb alone and in combination with insecticides*

Materials used[a]		Percentage[b] of bright fruits when the variety and date of application was as indicated					
Amount of zineb		Ruby Red grapefruit			Hamlin orange		
(lb./100 gal.)	Insecticide	July 2	July 16	Aug. 3	July 2	July 16	July 30
0	None (control)	19.7	15.6	11.4	88.5	80.5	57.9
0	Oil-parathion	7.9	33.7	49.4	79.2	46.9	65.7
0.65	Oil-parathion	100.0	94.9	95.2	100.0	99.6	99.5
0.65	None	97.4	99.0	94.8	99.3	100.0	100.0
0.975	None	99.7	97.9	96.1	99.3	100.0	100.0
1.30	None	98.5	97.8	98.2	99.6	100.0	100.0

[a] Zineb was used as a 65% formulation. Oil (as a 90% formulation) was applied at 0.70 gal. per 100 gal. and parathion (as a 15% formulation) at 0.15 lb. per 100 gal.

[b] Based on 200–300 fruits per treatment. Records for grapefruit were made October 25, 1956, and those for oranges on October 30, 1956.

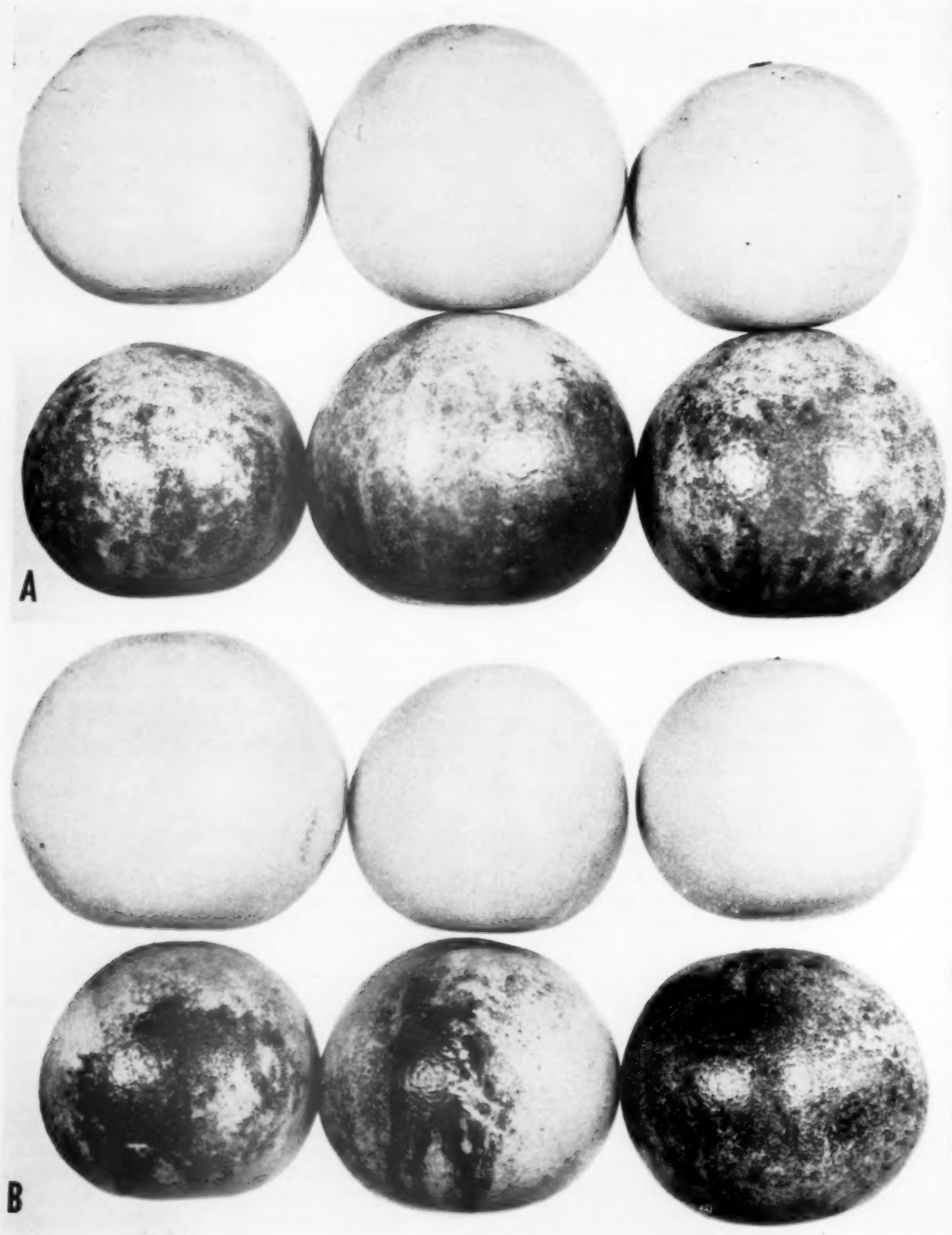


FIG. 2. A) Duncan grapefruit sprayed on July 17, 1956. Fruits in the first row were sprayed with oil-parathion plus 0.65 lb. zineb/100 gal., and those in the second row were sprayed with oil-parathion. B) Ruby Red grapefruit. Fruits in row 3 were sprayed with zineb on July 16, and those in row 4 were from the nonsprayed control.

brunneo-atrum. If these reports are confirmed, the general association of rust mites with these diseases needs to be explained.

Grove observations and terminal counts have shown rust mites to be present on some trees sprayed with zineb, but the infestations were very low 7 months following treatment. Adjacent trees, either not sprayed or treated with oil-parathion, had high rust mite populations. Yet no reports have been found in the literature concerning the miticidal effects of zineb. In the laboratory, grapefruit infested with rust mites were placed under a dissecting microscope and then sprayed with zineb (0.65 lb./100 gal.). The mites were motionless until the fruit dried, and then they began to crawl. These mites were observed for 5 hours; the spray did not appear to have any deleterious effects during this period.

The apparent control of rust mites by zineb has not been adequately explained,[4] nor have the field correlations between rust mites and previously mentioned fungus diseases. Several possibilities should be considered: 1) that rust mites are chemically destroyed or repelled by zineb, 2) that rust mites may feed on fungi and/or algae that are destroyed by zineb, or 3) that rust mites do not produce symptoms in plants that perhaps have been physiologically altered by the application of zineb.

The continual use of sulfur for the control of russet has created complex problems in the culture of citrus in Florida. That this disease has been controlled by a single summer application of zineb rather than by sulfur is of significance to the Florida citrus industry in several different ways. One of the main objections to the use of sulfur is that it brings about marked lowering of the pH in both surface soil and subsoil (14); this leads to adverse effects on availability of certain essential materials. Another facet is the possibility of toxicity of sulfates to citrus (6).

Benefits from the use in summer of zineb instead of sulfur are 1) a reduction in the number of applications, 2) a reduction in the deleterious effect of repeated sulfur applications on entomogenous fungi (4,5), 3) less damage to beneficial insect parasites and predators (9,10), and 4) smaller quantities of inert residues that appear to be an important factor in the increase of scale infestations (5).

CITRUS EXPERIMENT STATION
UNIVERSITY OF FLORIDA
LAKE ALFRED, FLORIDA

[4]Since this paper was written, the writer has encountered Tisdale and Flenner's paper (Contribs. Boyce Thompson Inst. **16**: 303-311. 1951) where some derivatives of the dithiocarbamates are reported to possess certain insecticidal properties. The suppression of mites by zineb is reported by D. R. King and D. W. Rosberg (Control of Tetranychus hicoriae McG. on pecans. J. Econ. Entomol. **49**: 404-405. 1956).

LITERATURE CITED

1. ASHMEAD, W. H. 1879. Injurious and beneficial insects found on the orange trees of Florida. Can. Entomologist **11**: 159-160.
2. BITANCOURT, A. A. 1940. A mancha de acaro ou falsa ferrugem das laranjas. (In Portuguese). Biologico **6**: 189-192.
3. FAWCETT, H. S. 1936. Citrus diseases and their control. McGraw-Hill, New York. 656 p.
4. FISHER, F. E. 1951. An Entomophthora attacking citrus red mite. Florida Entomologist **34**: 83-88.
5. FISHER, F. E., AND J. T. GRIFFITHS, JR. 1951. The fungicidal effect of sulfur on entomogenous fungi attacking purple scale. J. Econ. Entomol. **43**: 712-718.
6. HAAS, A. R. C., AND E. E. THOMAS. 1928. Effect of sulfate on lemon leaves. Botan. Gaz. **86**: 345-354.
7. HUBBARD, H. G. 1885. Insects affecting the orange. U. S. Dept. Agr., Div. Entomol. Bull. 1, p. 9-13.
8. MCALPINE, D. 1899. Fungus diseases of citrus trees in Australia and their treatment. J. Dept. Agr. Victoria. 132 p.
9. MUMA, M. H. 1956. Insect parasitism and related biological factors as concerned with citrus insect and mite control. Florida Agr. Expt. Sta. Ann. Rept. (In press.)
10. MUMA, M. H. 1956. Predators and parasites of citrus mites in Florida. 10th Intern. Congr. Entomol. Proc. (In press.)
11. ROLFS, P. H. 1905. Report of committee on diseases of citrus. Proc. 18th Ann. Meeting Florida State Hort. Soc., p. 29-32.
12. TANAKA, S., AND S. YAMADA. 1952. Studies on the greasy spot (black melanose) of citrus. I. Confirmation of the causal fungus and its taxonomic study. (In Japanese with English summary.) Hort. Div., Natl. Tokaikinki Agr. Expt. Sta. (Japan) Bull. **1**: 1-15.
13. THOMPSON, W. L., C. R. STEARNS, JR., AND W. A. SIMANTON. 1952. Combined control of scale insects and mites on citrus. Florida Agr. Expt. Sta. Ann. Rept., p. 167-169.
14. THOMPSON, W. L., I. W. WANDER, F. E. FISHER, AND J. T. GRIFFITHS, JR. 1952. The use of sulphur in citrus groves. Citrus Mag. **15**: 28-30.
15. WINSTON, J. R. 1921. Tear-stain of citrus fruits. U. S. Dept. Agr. Bull. 924, 12 p.
16. YOTHERS, W. W., AND A. C. MASON. 1930. The citrus rust mite and its control. U. S. Dept. Agr. Tech. Bull. 176, 56 p.

PATHOGENICITY OF IDRIELLA LUNATA ON STRAWBERRY [1]

Paul E. Nelson [2]

SUMMARY

Idriella lunata was found to be pathogenic to strawberry. Under the conditions of various experiments performed, the fungus caused rootlet degeneration and tip killing of the large adventitious roots. It also produced black sunken lesions on the adventitious and branch roots. Hyphae and chlamydospore masses of *I. lunata* commonly developed in the outermost cell layers of the living root cortex; in killed rootlets, root tips, and lesions, they developed in all the tissues of the root. Xylem vessels often were plugged by chlamydospore masses and tissue degeneration products. Phloem tissues showed extensive disintegration.

Under field conditions, *I. lunata* has been isolated only from the roots of strawberry plants in areas where root deterioration was extensive. Under greenhouse conditions it grew on or in the roots of tomato (*Lycopersicon esculentum* var. *commune*), pepper (*Capsicum frutescens* var. *grossum*), carrot (*Daucus carota* var. *sativa*), lettuce (*Lactuca sativa* var. *longifolia*), Honeydew and Baby Persian melons (*Cucumis melo*), and *Potentilla delphinensis*. Except for the melons, it caused no apparent injury to the roots of these plants. The roots of both Honeydew and Baby Persian melons were infected and showed evidence of injury.

Under greenhouse conditions the fungus did not cause pre-emergence or postemergence damping-off of strawberry seedlings. Under conditions favorable for the development of strawberry leaf spot, caused by *Mycosphaerella fragariae*, *I. lunata* did not attack the foliage of strawberry.

The root deterioration disease of strawberry, as it was found in certain areas of California, is believed to be caused primarily by infection by *I. lunata* followed by invasion of the root system by low-grade pathogens or secondary invaders that inhabit the rhizosphere.

Root diseases responsible for the premature decline and unfruitfulness of strawberry plantings are troublesome nearly everywhere the strawberry is grown. Various names such as black root, black root rot, root rot, and root weakness have appeared in the literature and are somewhat descriptive of the trouble (1, 7, 9, 12, 15, 16, 18). In California, it seems appropriate to consider the problem of root deterioration as 1 general disease, admitting the possibility of numerous causes, rather than to relate it specifically to black root rot as described elsewhere. Evidence of root deterioration is not always apparent aboveground. The principal effect on the plant is disintegration of the outer protective and storage tissues of the main supporting roots, lack of fine absorbing rootlets, and weakened top growth to a degree corresponding to the extent of the root damage. This paper presents a detailed study of a previously unidentified fungus pathogen as one of the factors contributing to strawberry root deterioration.

THE ORGANISM.—*Idriella lunata* Nelson et Wilhelm (11) was isolated from roots of infected strawberry plants taken from a planting in the Santa Clara valley of California where root deterioration was manifest in a severe form. This field was planted to the variety Twentieth Century in May, 1950. The plants began to fail in large distinct circular areas late in the summer of 1950. The following year the planting became unprofitable.

Plants removed from the field late in the summer of 1950 exhibited a completely dead initial root system with little evidence that it had ever started to grow (Fig. 1,A). The new adventitious roots produced since planting displayed extensive blackening, which was more severe at the root tips than near the crown. Microscopic examination of some of these roots in the laboratory after they had been cleared for several days in 40 per cent lactophenol revealed characteristic masses of chlamydospores in the outer cortical tissues of these roots (Fig. 1, C, D).

Of the several isolation techniques employed, surface sterilization of root pieces in a 1:9 dilution of Clorox (5.25% sodium hypochlorite) for 10–60 minutes followed by blotting on sterile blotters and plating on various natural media gave the best results. Surface sterilization is necessary because the fungus grows

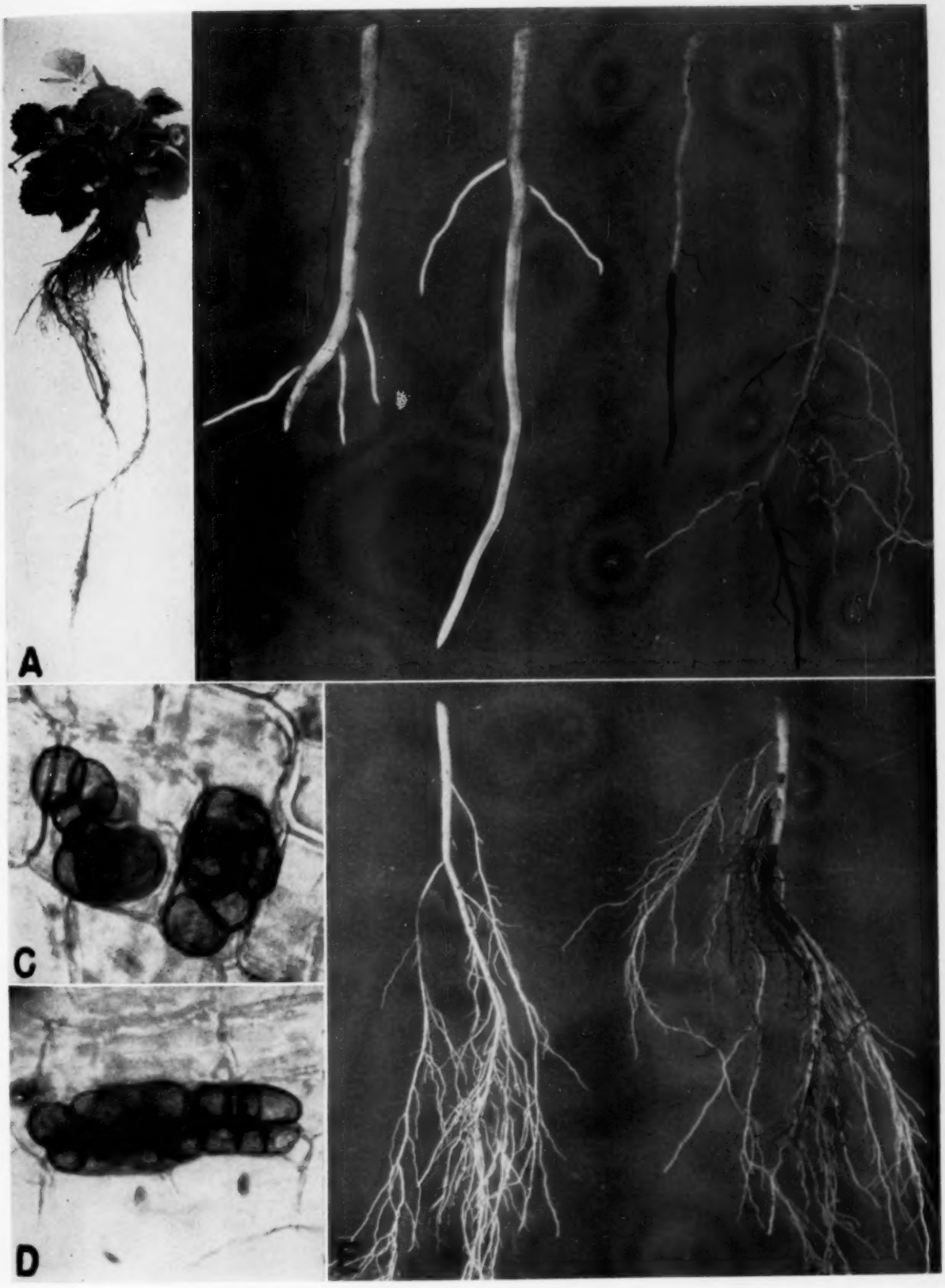
[1] Accepted for publication March 10, 1957.

A portion of a thesis submitted to the Graduate Division of the University of California at Berkeley in partial satisfaction of the requirements for the degree of Doctor of Philosophy.

The author expresses his sincere gratitude to Dr. S. Wilhelm for the encouragement, guidance, and helpful suggestions given throughout this work.

[2] Former Research Assistant, University of California at Berkeley; now Assistant Professor of Plant Pathology, Cornell University, Ornamentals Research Laboratory, Farmingdale, New York.

FIG. 1. A) Strawberry plant grown for 18 months in soil infested with root rot organisms. B) Large adventitious strawberry roots from a check plant (left) and from an inoculated plant (right). The latter shows tip die-back on both roots and rootlets. C,D) Characteristic chlamydospore masses formed in the outer cortical tissues of roots from naturally infected strawberry plants. C, 650×. D, 580×. E) Large adventitious strawberry roots from a check plant (left) and from an inoculated plant (right). The latter shows a black lesion and attached dead feeder rootlets.


A
C
D

slowly and is readily overgrown by faster growing saprophytes and root-surface bacteria. The chlamydospore masses, which are often readily visible in roots taken from the field, are somewhat resistant to the Clorox treatment; following such treatments, the fungus was obtained readily in pure culture either by single sporing from conidia produced directly on the roots or by transfer of hyphal tips. Of the natural media, wheat and barley straws supported excellent growth and favored chlamydospore development. These straws, first sterilized by fumigation with propylene oxide in tightly closed jars, were incorporated into 2 per cent plain agar as described by Hansen and Snyder (6).

Chlamydospore masses also form in thoroughly washed roots placed in sterile water or in sterile moist sand (19). These methods offer advantages in that subisolation can be made from the specific root area containing the chlamydospore masses but are disadvantageous in that exposed strawberry roots often turn dark rapidly obscuring the chlamydospore masses.

Temperature has a profound effect upon this fungus, affecting colony characteristics, sporulation, and gross morphology. Germinating conidia of the fungus were transferred to 3 separate, equidistant positions in petri dishes containing approximately 20 ml of potato-dextrose agar. Four such plates were used for each of 2 different isolates of the fungus for each temperature, giving a total of 12 colonies of each isolate for comparative study. The young colonies were allowed to grow at room temperature for 2–3 days after being transferred to petri dishes. The dishes were then placed in moist chambers lined with paper towels saturated with a 5 per cent solution of copper sulfate to suppress the growth of contaminants. These were placed in unlighted constant-temperature incubators held at temperatures ranging from 7° to 34°C in 3° intervals. The diameter of each colony was measured at 3-day intervals for a period of 34 days, the duration of the experiment.

The optimum temperature range for vegetative growth in culture was 19°–22°C. Only a trace of growth occurred at 7°C, and growth was suppressed at 34°C.

Optimum conditions for sporulation in culture were obtained by growing the fungus out of doors in a shaded rack on the west side of a building. Light and fluctuating temperature proved to be necessary factors for sporulation.

PATHOGENICITY.—*I. lunata* was tested for its ability to cause damping-off of strawberry seedlings by planting seed in pots of steam-sterilized soil and watering the seed with a spore suspension. The seedlings were checked for damping-off over a 3-month period. Stands of seedlings in both checks and inoculated series were equally good. Under the conditions of this experiment, *I. lunata* did not cause either pre-emergence or postemergence damping-off of strawberry seedlings.

The effect of *I. lunata* on foliage of strawberry plants was studied by comparing the effect of 2 isolates with that of *Mycosphaerella fragariae* (Tul.) Lindau. Test plants, which had been in a moist chamber for 2½ days, were inoculated with a spore suspension by the method described by Fall (3). In some instances both upper and lower leaf surfaces were inoculated, whereas in others only the upper or the lower surface was inoculated. The test plants were then placed in a moist chamber for 2 more days and then placed in a greenhouse. In 10 days, leaf spots appeared on plants inoculated on the lower leaf surface with spores of *M. fragariae*. This fungus was recovered in culture from these spots. None of the other test plants showed any leaf spots after 2 months, the duration of the experiment. Under the conditions of this experiment, *I. lunata* did not cause a leaf spot of strawberry.

Three different methods of inoculation were used to test the pathogenicity of *I. lunata* on the strawberry root system. In the first of these experiments, the inoculum was grown on a previously sterilized mixture of dried tomato stem pieces, coarse sand, and water and incorporated into steam-sterilized field soil before planting. In the second experiment, the roots of test plants were dipped in a spore suspension for 5 minutes just prior to planting in steam-sterilized field soil. In the third experiment, test plants were planted in steam-sterilized field soil and then watered with a spore suspension immediately after planting. Plants used in these tests were either grown from seed in sterilized soil or were runners that had been rooted in pots of sterilized soil. The same pattern of root killing occurred in all 3 experiments, with the principal difference being in the length of time necessary for root damage to appear. Root damage appeared much sooner in the second and third experiments than it did in the first.

The results reported here were obtained from 2 experiments in which roots were dipped in a spore suspension just prior to planting. Washed root systems of strawberry seedlings were dipped in a spore suspension and planted in steam-sterilized field soil in 6-in. pots for the first experiment. Eight weeks after inoculation, check plants still had healthy seedling root systems whereas inoculated plants showed extensive root killing. The root killing appeared to be confined mainly to the roots actually inoculated; the plants survived by putting out new adventitious roots, which at this time were not seriously involved. After 15 weeks, many of the large adventitious roots on inoculated plants exhibited dark sunken lesions (Fig. 1.E). After 7½ months, root growth on inoculated plants was very poor. There was a great deal of rootlet killing, and lesions were evident on both large and small roots. Many of the large adventitious roots also exhibited dieback from the tips (Fig. 1.B). Roots showing lesions or tip killing, and some that were apparently unaffected, were cultured; the fungus was readily recovered in culture from all of them.

In the second experiment, the root systems of 2-month-old runner plants rooted in sterilized greenhouse soil were washed as free of soil as possible, trimmed

until the remaining roots were about 3 in. long, dipped in a spore suspension for 5 minutes, and planted in steam-sterilized field soil in 6-in. pots. One week after inoculation and thereafter at 2-week intervals for a total of 7 weeks, plants were removed for culturing and examination. After 3 weeks, microscopic examination revealed some rootlet killing and chlamydospore masses scattered throughout both white healthy-appearing areas and the dead and dying areas of the rootlets (Fig. 2,E). After 5 weeks, inoculated plants exhibited an abundance of dead feeder rootlets; in fact, few white feeder rootlets were present. Some of the new adventitious roots were killed from the tip back (Fig. 1,B), and chlamydospores were present in the dead and dying areas of the root system. After 7 weeks, black sunken lesions had begun to form on the large adventitious roots (Fig. 1,E) and chlamydospores had formed throughout the root system and in the lesions. The experiment was continued for an additional 6 weeks, and the root systems of inoculated plants continued to deteriorate.

The results of these experiments indicate that infection takes place through the root tip. The roots and rootlets always appear to be killed from the tips back. This is particularly evident on the large adventitious roots (Fig. 1,B).

Pathological anatomy of infected roots.—Root samples were collected at various times from artifically inoculated strawberry plants grown in 6-in. pots of steam-sterilized field soil. The samples were washed free of soil, fixed in Rawlins' alcohol-formalin-acetic acid solution No. 1 (13) for 48 hours or longer, dehydrated according to the *n*-butyl alcohol schedule (13), and embedded in paraffin. Prior to the sectioning, embedded roots were softened for 48 hours or longer in small amounts of a solution composed of 90 ml of 1 per cent aqueous solution of Dreft (sodium lauryl sulfate) and 10 ml of glycerol. Sections were cut on a rotary microtome at 10 μ and stained according to a modified Johansen's quadruple stain schedule (8, p. 88).

Root samples also were collected, fixed in a similar manner, and cleared in 40 per cent lactophenol for 2–6 weeks. Cleared roots were mounted unstained in lactophenol.

Studies of prepared sections and cleared rootlets showed that the fungus was primarily in the cortical tissue of the roots (Fig. 2,A). Blackened feeder rootlets attached to a lesion area of a large adventitious root when cleared showed typical chlamydospore masses of *I. lunata* in the steles of these roots. In 1 instance, chlamydospores had formed in a young vessel and the annular secondary thickenings of the vessel had formed indentations in the walls of the chlamydospore (Fig. 2,B). Under favorable conditions, the fungus grew in the tissues of the stele as well as the cortex.

In the larger adventitious roots, the fungus was present also in the cortex, and chlamydospores were formed in the outermost cortical cell layers. In areas where lesions had formed, it appeared to pervade the stelar tissues as well as those of the cortex (Fig. 2,C). Lesions formed on the main adventitious roots adjacent to previously killed rootlets (Fig. 1,E). It appears that whenever *I. lunata* attacks a large adventitious root at any point other than the tip, it probably enters through a lateral rootlet and a lesion subsequently is formed. Anatomical studies of a lesion showed collapsed cortical (Fig. 2,D) and phloem tissues and plugged xylem vessels. Plugging of the xylem vessels may be due either to the formation of chlamydospores in the vessels (Fig. 2,B) or to infiltration of the vessels with various substances resulting from the deterioration of the root. The initiation of a lesion and the subsequent tissue degeneration result in the elimination of this adventitious root from the functioning root system; therefore extensive lesion formation results in reduced efficiency of the root system and a gradual decline of the plant.

Host range.—Seedlings of tomato (*Lycopersicon esculentum* Mill. var. *commune* Bailey), pepper (*Capsicum frutescens* L. var. *grossum* Bailey), carrot (*Daucus carota* L. var. *sativa* DC.), lettuce (*Lactuca sativa* L. var. *longifolia* Lam.), celery (*Apium graveolens* L. var. *dulce* Pers.), Honeydew and Baby Persian melons (*Cucumis melo* L.), snapdragon (*Antirrhinum majus* L.), *Potentilla nepalensis* Hook., and *P. delphinensis* were used as test plants. Seedlings of each test plant were inoculated by dipping their roots in a spore suspension and then were planted in steam-sterilized field soil; another group of seedlings was planted in steam-sterilized field soil infested with inoculum grown on the mixture of sand and tomato stems. A suitable check series was set up at the same time.

After 8 weeks, plants were removed from each series for examination and culturing. The inoculated plants showed no symptoms and they did not differ from their checks in root growth. The fungus was recovered in culture only from the roots of the Honeydew melon and Baby Persian melon. After 15 weeks, the 2 melons showed poorer root growth than did the checks, and chlamydospore masses of *I. lunata* were abundant in the roots. Other inoculated plants in the experiment did not differ from their controls in root growth. Cultures were made from the roots of all plants. The fungus was not recovered from the roots of celery, snapdragon, and *Potentilla delphinensis*. It was recovered from the roots of tomato, pepper, carrot, lettuce, and *P. nepalensis* only in instances where, prior to culturing, the roots were rinsed in sterile water but not surface sterilized in Clorox and only from the inoculation series in which the soil had been infested with the mixture of sand and tomato stems. *I. lunata* was recovered from the roots of Honeydew melon and Baby Persian melon from the series inoculated with a spore suspension and from the series in which soil was infested with the mixture, whether or not surface sterilization with Clorox was used.

Discussion.—Hyphae and chlamydospore masses of

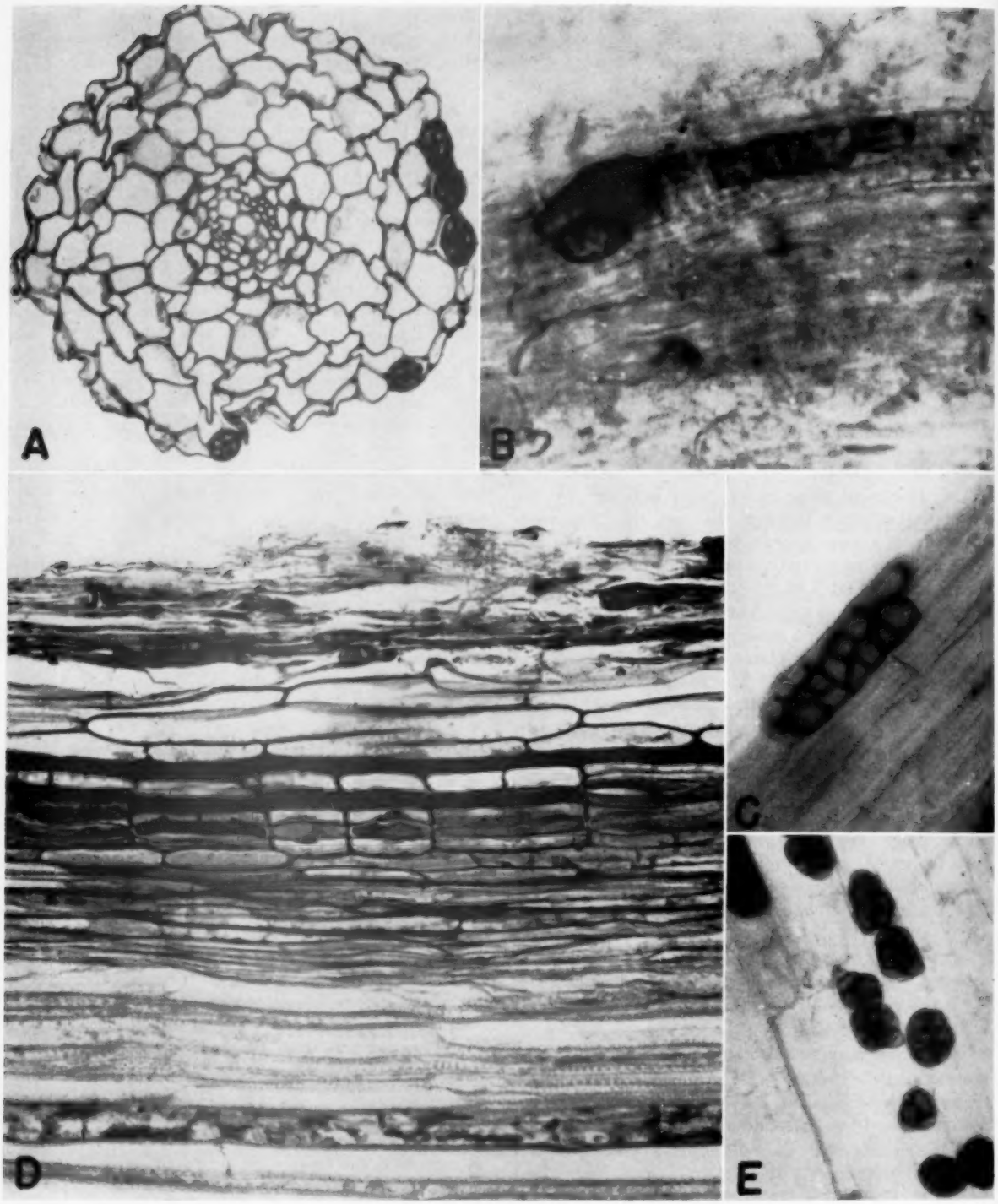


FIG. 2. A) Transection of a small strawberry feeder rootlet, showing chlamydospores in the outermost cell layers of the cortex (395×). B) Chlamydospore formed in the stelar tissues of a cleared section of an inoculated strawberry feeder rootlet. Notice the indentations formed in the chlamydospore walls by the annular secondary thickenings of the young vessel (1220×). C) Chlamydospore formed in the stelar tissues of a large adventitious strawberry root with the cortex stripped away (435×). D) Longitudinal section of a lesion on a large adventitious strawberry root showing collapse of the cortical tissues containing chlamydospores (310×). E) Chlamydospore masses and hyphae of *Idriella lunata* formed in the cortical tissue of an inoculated strawberry root (335×).

I. lunata commonly develop in the outermost cell layers of the root cortex. In lesions or in killed root tips, they pervade all the tissues of the root. This complete invasion of root tissues in these areas leads to their ultimate destruction and to a gradual decline of the plant.

The typical chlamydospore masses of *I. lunata* formed in the root tissues of infected plants may be important in the survival of the fungus in soil. These chlamydospores are undoubtedly the structures best suited to carry the fungus through periods of adverse environmental conditions. During the course of the isolation of *I. lunata* from root samples of field-grown strawberries, these chlamydospores were resistant to treatment in 10 per cent Clorox for as long as 60 minutes.

I. lunata may be an important factor in causing root deterioration of strawberry, not only because of its initial injury to roots but also because it may prepare these roots for invasion by saprophytes of the rhizosphere microflora that may become definitely pathogenic after admittance. The concept of a primary parasite preparing plant tissues for invasion by weak secondary parasites and saprophytes is one that might be considered in attempting to explain the root-deterioration disease of strawberry. A number of examples of such phenomena are found in the literature relating to both root and foliage diseases. Hansen (5), working with the pink-root disease of onion (caused by *Pyrenochaeta terrestris* (Hans.) Gorenz et al), reported that saprophytic Fusaria often entered and aggravated the disease. McKeen (10) reported that *Phialophora radicicola* Cain may be of primary importance in corn root degeneration by enabling parasites and saprophytes not able to gain entrance into non-infected tissues to gain entrance into corn roots after they were infected by this fungus. Garrett (4) states that "Once a habitat has been colonized by the pioneer, autogenic changes prepare the way for colonization by following organisms, which, sooner or later, displace the original pioneer. Infection of a plant root by a parasitic fungus and the subsequent development of weak secondary parasites and saprophytes in the tissues is thus seen as an example of plant succession, a phenomenon already thoroughly familiar to ecologists studying surface vegetation." Various investigators (2; 14, p. 219; 17) have found that rust pustules on the foliage of various host plants are often invaded by secondary organisms, such as *Fusarium* spp. and *Botrytis* sp., which may advance into the surrounding host tissues and bring about their death. These same workers also report that these secondary fungi were apparently unable to invade healthy host tissues but could only enter through the rust lesions.

The root deterioration disease of strawberry, as it is known in California, may be due to initial infection by *I. lunata* followed by invasion of the root system by low-grade pathogens or secondary invaders that inhabit the rhizosphere.

Department of Plant Pathology
University of California
Berkeley 4, California

LITERATURE CITED

1. Berkeley, G. H. 1944. Root-rots of certain non-cereal crops. Botan. Rev. **10:** 67-123.
2. Dimock, A. W., and K. F. Baker. 1951. Effect of climate on disease development, injuriousness, and fungicidal control, as exemplified by snapdragon rust. Phytopathology **41:** 536-552.
3. Fall, Joan. 1951. Studies on fungus parasites of strawberry leaves in Ontario. Can. J. Botany. **29:** 299-315.
4. Garrett, S. D. 1952. The soil fungi as a microcosm for ecologists. Sci. Progr. **40:** 436-450.
5. Hansen, H. N. 1929. Etiology of the pink-root disease of onions. Phytopathology **19:** 691-704.
6. Hansen, H. N., and W. C. Snyder. 1947. Gaseous sterilization of biological materials for use as culture media. Phytopathology **37:** 369-371.
7. Hildebrand, A. A., and P. M. West. 1941. Strawberry root rot in relation to microbiological changes induced in root rot soil by the incorporation of certain cover crops. Can. J. Research, Sect. C **19:** 183-198.
8. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.
9. Katznelson, H., and L. T. Richardson. 1948. Rhizosphere studies and associated microbiological phenomena in relation to strawberry root rot. Sci. Agri. **28:** 293-308.
10. McKeen, W. E. 1952. Phialophora radicicola Cain, a corn rootrot pathogen. Can. J. Botany **30:** 344-347.
11. Nelson, P. E., and S. Wilhelm. 1956. An undescribed fungus causing a root rot of strawberry. Mycologia **48:** 547-551.
12. O'Brien, D. G., and E. J. M'Naughton. 1928. The endotrophic mycorrhiza of strawberries and its significance. West Scot. Agr. Coll. Research Bull. 1, 32 p.
13. Rawlins, T. E. 1933. Phytopathological and botanical research methods. John Wiley and Sons, New York. 156 p.
14. Stewart, F. C. 1899. Notes on various plant diseases. N. Y. (Geneva) Agr. Expt. Sta. Bull. **164:** 207-221.
15. Strong, F. C., and Miriam C. Strong. 1931. Investigations on the black root of strawberries. Phytopathology **21:** 1041-1060.
16. Truscott, J. H. L. 1934. Fungous root rots of the strawberry. Can. J. Research **11:** 1-17.
17. Walker, J. C. 1921. Rust of onion followed by a secondary parasite. Phytopathology **11:** 87-90.
18. West, P. M., and A. A. Hildebrand. 1941. The microbiological balance of strawberry root rot soil as related to the rhizosphere and decomposition effects of certain cover crops. Can. J. Research, Sec. C **19:** 199-210.
19. Wilhelm, S. 1956. A sand-culture technique for the isolation of fungi associated with roots. Phytopathology **46:** 293-295.

The Morphology of the Sclerotium of Claviceps paspali.[1] ROBERT B. STEWART.[2] The sclerotia of *Claviceps paspali* Stevens & Hall are reported by Brown[3] as "globular in shape, 2–4 mm in diameter, irregularly roughened on the surface, yellowish gray in color; the interior is homogenous in structure and contains a considerable quantity of oil." Apparently there have been no further investigations of the structure of the sclerotium.

In the course of investigations of ergot of Dallis grass in south central Texas, permanent mounts were made of fruiting sclerotia of *C. paspali*, the causative agent of ergot in this locality. It was noted that the sclerotia showed certain morphological characters that have not been reported previously.

The sclerotia were collected near College Station, Texas, on May 15, 1954, and were germinating naturally when collected. The sclerotia were killed in a standard formalin-aceto-alcohol solution and embedded in paraffin blocks by the butyl alcohol method. The material was then sectioned with a rotary microtome and stained with a modified Conant's quadruple stain.[4]

The observations made on this material show that the sclerotium is considerably more complex than indicated by Brown and is actually composed of 3 distinct layers that become differentiated as the sclerotium reaches maturity. These layers are designated as the surface layer, transitional layer, and germ layer (Fig. 1). Development of the outer layers can be attributed to weathering; they may be considered as protective devices that preserve the vitality of the germ layer of the sclerotium, somewhat analogous to the seed coat or pericarp of higher plants. All 3 layers are of the same origin, i.e. a mass of undifferentiated hyphae. The surface layer differs from the others only in that it is composed of dead cells. The transitional layer stains differently than the germ layer, a possible indication of physiological differentiation. Young sclerotia do not show these layers but are homogenous throughout.

The surface layer is composed of dead fungal cells that are somewhat shrunken and distorted as compared to those of the interior, giving the sclerotium the roughened, cracked, irregular appearance that characterizes this species. The cracks in the surface layer do not penetrate into the lower layers but stop short of the transitional layer. The surface layer is of varying depth, depending largely upon the degree of weather-

Paper No. T. A. 2579 Texas Agricultural Experiment Station. Published with the approval of the Director of the Texas Agricultural Experiment Station.

Work reported forms a portion of a thesis to be submitted to the Graduate School, Agricultural and Mechanical College of Texas, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Assistant Professor, Department Plant Physiology and Pathology, Agricultural and Mechanical College of Texas.

[3] Brown, H. B. 1916. Life history and poisonous properties of Claviceps paspali. J. Agr. Research 7: 401-406.

[4] Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, New York.

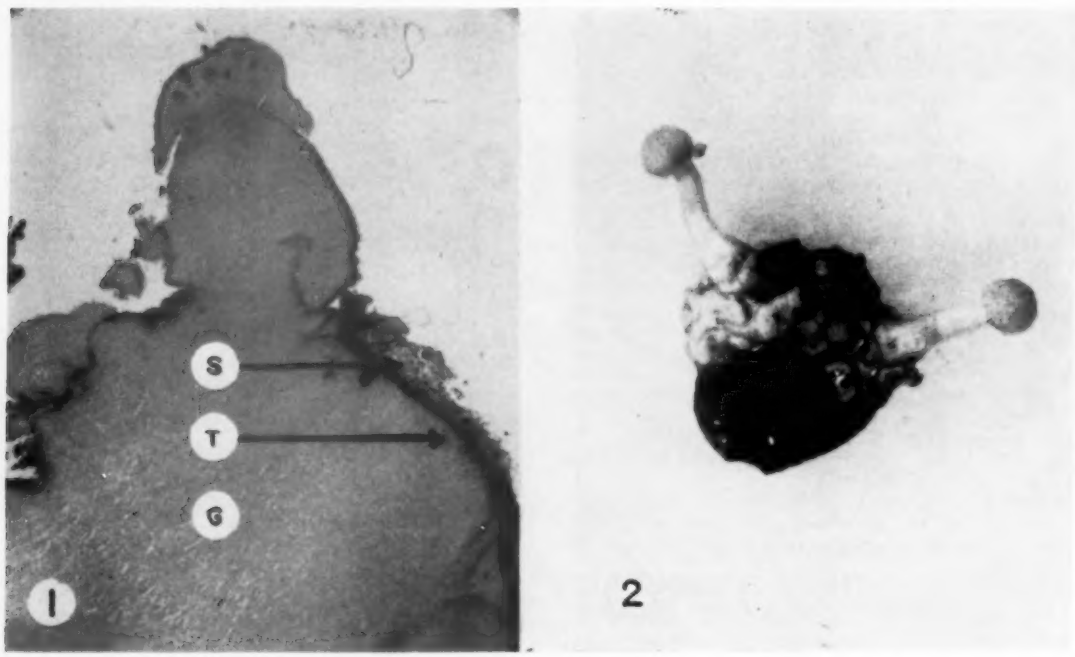


FIG. 1–2. Fig. 1. Fruiting structure emerging from a sclerotium of *Claviceps paspali*. (S) Surface layer. (T) Transitional layer. (G) Germ layer (× 26).—Fig. 2. A mature fruiting sclerotium of *C. paspali*. Note the outer layers are folded back where the fruiting bodies have emerged (× 10).

ing to which the sclerotium has been exposed and upon the degree of erosion of the outer cells. It is usually much thicker than the transitional layer. The surface layer is thin in areas that are externally protected, as where the lemma and palea adhere to the sclerotium. Although the depth of the surface layer is too variable to be considered in any way diagnostic, it is often about 240 μ thick.

The transitional layer is regular in outline and about the same thickness (15μ) throughout. It consists of only a few slightly differentiated cells and is somewhat intermediate in appearance, having certain characteristics of both the surface and germ layers. In sclerotial sections stained with modified Conant's stain, the transitional layer shows a distinct affinity for safranin whereas the germ layer stains green and the surface layer green to pink. Its affinity for safranin suggests the presence in the transitional layer of some compound similar to suberin. The cells in the periphery of the transitional layer are morphologically similar to the shrunken and dried cells of the surface layer, whereas the innermost cells of the transitional layer do not appear morphologically distinct from those of the germ layer. Within the transitional layer, there is a gradation in affinity for safranin; the cells in the periphery stain a deeper red than do the inner cells. Where cracks in the surface layer occur, there is a noticeable intensification of the red color at the point where the fissure approaches the transitional layer.

The germ layer of the sclerotium is the vital portion and constitutes approximately 90 per cent of a sclerotium. The cells of this germ layer are irregular in size and shape, densely compacted, and interwoven. There is, as previously reported by Brown, a considerable quantity of oil globules throughout this tissue, but these are not apparent in the outer layers.

The stipe is initiated in the germ layer. During stipe formation, hyphae of certain areas of the germ layer form small rounded masses that push their way through the transitional and surface layers to the exterior. Figure 2 shows the surface and transitional layers as they have been ruptured and folded back by the emergence of the fruiting bodies.

It was noted that when the immature fruiting bodies or "buttons" were mechanically removed, the sclerotium readily developed new ones. This process apparently can be repeated until the food reserve of the sclerotium is exhausted.—Department of Plant Physiology and Pathology, Agricultural and Mechanical College of Texas, College Station, Texas.

An Improved Method of Inoculating Barley Leaves with Rhynchosporium secalis.[1] W. P. Skoropad.[2] The initial stages of barley infection by *Rhynchosporium secalis* (Oud.) J. J. Davis are favored by a relative humidity of about 95 per cent and a temperature of 15°–18°C during the first 48 hours after inoculation.[3] Special conditions also are required for the production of conidia in nature[3] and in culture.[4] The following are simple methods for inducing sporulation and for securing a high relative humidity during the early stages of incubation.

Dormant mycelium on barley leaves floated on water in a Petri dish sporulates abundantly within 48 hours at 10°C. Young cultures of this fungus also sporulate readily on various agar media at 15°–20°, provided these media are fairly soft. Malt-yeast medium containing 1 per cent agar is satisfactory because it supports vigorous growth of the culture for a long time.

To inoculate plants, conidia from either of the above sources are mixed with soft agar, and this mixture is placed on a selected part of a barley leaf by means of a scalpel or a stiff camel's-hair brush. A scald lesion develops for only a few millimeters around the point of inoculation; therefore, its area can be controlled by the extent to which the inoculum is spread.

After inoculation, a high relative humidity can be maintained by covering the plants with a polythene bag in which the air is saturated with moisture. The bag may be unnecessary, however, if the inoculated plants are sprayed with water at intervals of 3–4 hours. By either of the above means, the mixture of agar and conidia retains sufficient moisture to enable the fungus to penetrate the leaf and establish itself in the host tissue.

This method of inoculation makes it possible to predetermine the location and the extent of scald lesions on barley leaves. It is, therefore, especially useful in race studies because the number of lesions can be correlated with the number of spots that were inoculated. It is also valuable for tests designed to determine the effect of scald on yield of barley, since the area of leaf lesioning can be controlled.—Plant Pathology Laboratory, Edmonton, Alberta.

Fruit Decay and Twig Cankers on Peach. M. A. Smith and G. B. Ramsey.[1] During holding tests of South Carolina Elberta peaches in August, 1953 and 1956, a type of decay on fruit that did not resemble brown rot or Rhizopus rot was observed. The decayed areas were fairly firm and at first appeared as a brown surface discoloration; later, a white surface mold developed on affected areas. Patches of black fruiting bodies appeared on older lesions, and eventually the affected peaches became shrivelled and mummified. Examination disclosed the presence of pycnidia that

[1] Contribution No. 1583 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Associate Plant Pathologist, Edmonton.

[3] Caldwell, R. M. 1937. Rhynchosporium scald of barley, rye and other grasses. J. Agr. Research 55: 175-178.

[4] Schein, R. D., and J. W. Kerelo. 1956. Culturing Rhynchosporium secalis. Plant Disease Reptr. 40: 814-815.

[1] Respectively, Senior Pathologist and Principal Pathologist, Biological Sciences Branch, Agricultural Marketing Service, U. S. Department of Agriculture.

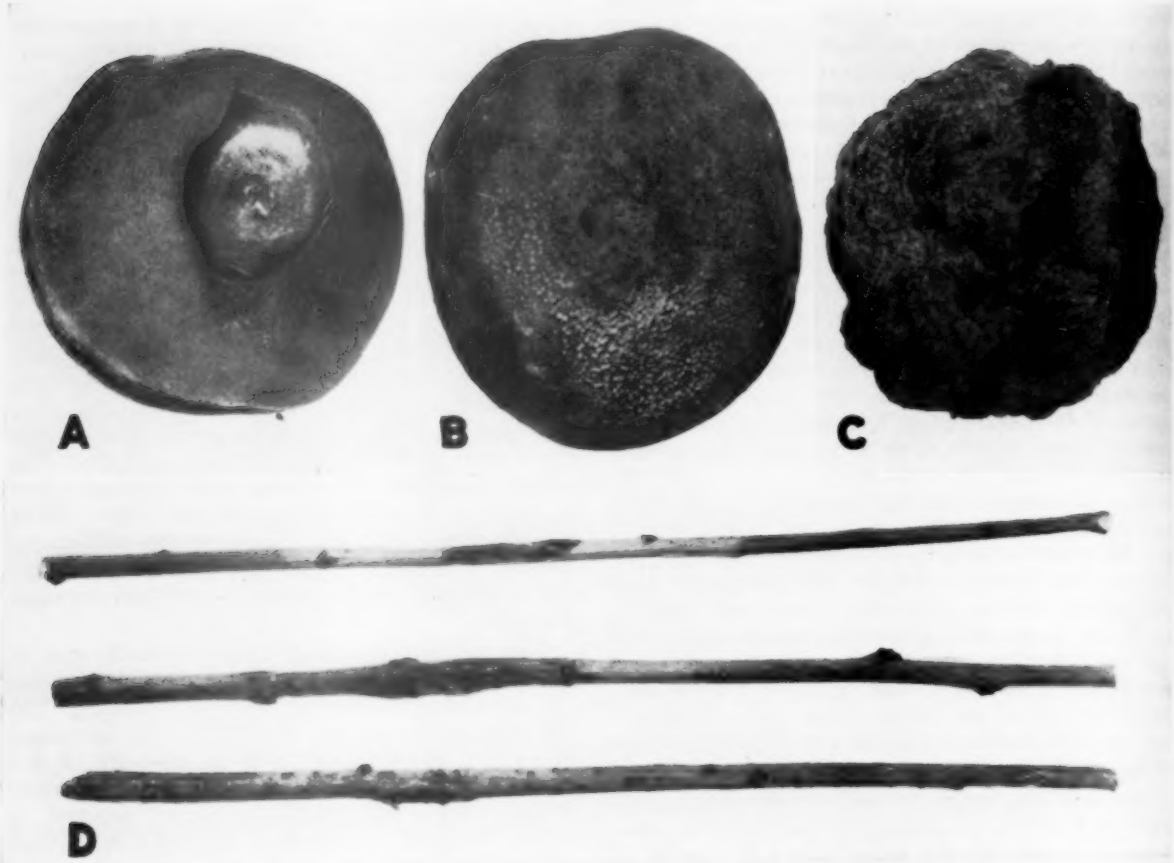


FIG. A-C) Decay on Elberta peaches inoculated with Phomopsis and then held at 78°–85°F for A) 7 days, B) 10 days, and C) 19 days. D) Cankers on Elberta peach twigs 70 days after inoculation with Phomopsis.

produced pycnospores and stylospores typical of *Phomopsis*. Pycnospores from 1-month-old potato-dextrose-agar cultures measured 2.0–4.1 × 6.2–9.8 μ (average 3.1 × 7.2 μ).

A series of inoculations was made on fruit held at different temperatures. Peaches were inoculated 1) by inserting a small amount of inoculum into small scalpel cuts and 2) by spraying nonwounded fruits with a suspension of pycnospores from a 1-month-old culture of the Phomopsis in sterilized water. All inoculated fruit incubated at temperatures of 45° F or of 60°–62° were observed for 17 days and those incubated at 78°–85° for 7 days. Average diameters of decayed areas on wounded inoculated fruit were 3 mm at 45°, 24 mm at 60°–62°, and 37.5 mm at 78°–85°. Of the inoculated nonwounded fruits, no infection developed at 45°, 16 per cent of the fruits held at 60°–62° were infected, and 100 per cent of those held at 78°–85° were infected. The average diameters of decayed areas on nonwounded fruits were 9 mm at 60°–62° and 12 mm at 78°–85°. Controls, which consisted of wounded and nonwounded fruits injured with sterile scalpel cuts or sprayed with sterilized water, remained free of the disease. The decay of the inoculated fruits held at 78°–85° is shown in Fig. 1, A, B, C.

Six twigs of 1-year-old Elberta peach trees were inoculated by introducing small portions of a 3-week-old culture into small scalpel cuts. The cuts were then covered with moistened cotton around which aluminum foil was wrapped. Noninoculated cuts made into twigs and similarly wrapped served as controls. Sixty days after inoculation, during which time the temperature ranged from 55° to 85°F, cankers were observed at the site of the various inoculations (Fig. 1, D). The twigs were removed 10 days later, and tissue platings were made from the cankers to potato-dextrose-agar plates and incubated at 80°F. Growth of the Phomopsis was evident within 3 days. After 10 days, the average diameter of the colonies was 30 mm. The controls remained free of the disease.

The inoculation tests along with the morphological studies of the fungus causing fruit rot indicate that it is the species of *Phomopsis* that causes the constriction disease of peach trees. The fungus is referred to in current literature as *Phoma persicae* Sacc. (*Diaporthe eres* Nits.), but presumably it should be transferred to *Phomopsis*.—Agricultural Marketing Service, U. S. Department of Agriculture, Chicago, Illinois.

Association Between Rhizoctonia and Yellow Coloration of Easter Lily Bulbs.[1] FRANK P. MCWHORTER.[2] The outer scales of typical commercial bulbs of *Lilium longiflorum* Thunb. var. Croft usually are blotched with a yellow pigment and marked with numerous microlesions and macrolesions of various colors. When pentachloronitrobenzene (PCNB) was used to treat "yearlings", the size planted to produce commercial bulbs, the chemical tended in several experiments to increase the growth of the commercial bulbs and to improve their appearance. A recent test of PCNB on stem bulblets, the size planted to produce yearlings, was made for us by Mr. Berger Dahlstrom, a commercial grower. This test consisted of generously dusting comparable units of stem bulblets with 75 per cent PCNB and planting them at Harbor, Oregon, where *Rhizoctonia solani* Kühn is a common constituent of the soil flora. The treatments and controls were replicated 5 times. In every case the yearlings from the untreated bulblets had the usual coloration whereas those from dusted bulblets appeared snow white. Yearlings from 4 of the treated lots were treated with PCNB and planted along with corresponding controls to follow the effects on commercial bulb production. The remaining yearlings from the treated lot and from the control were brought to Corvallis and analyzed for weight, root quality, and coloration.

The treated lot had yielded 47 bulbs with an average bulb weight of 36.3 g and had 937 roots, 39.7 per cent of which graded excellent. There was a trace of yellow pigment on 1 scale on each of 4 bulbs, and only 4 scales showed noticeable pigmentation. The untreated lot had yielded 49 bulbs with an average bulb weight of 29.3 g and had 917 roots, 15.6 per cent of which graded excellent. Every outside scale of every bulb was bright yellow; approximately 300 scales were strongly pigmented.

The greater weight of the yearlings from treated bulblets was directly proportional to the higher percentage of good roots. Whether the greater percentage of good roots was due principally to control of Rhizoctonia by PCNB was not determined. That the lack of yellow coloration, however, correlated directly with control of Rhizoctonia was definitely indicated by the abundance of Rhizoctonia on the yellow scales and its absence or near absence from the white.

On yellowed scales from lilies in this experiment and from many other Croft bulbs, there was a direct correlation among the abundance of Rhizoctonia, the number of microlesions, and the number of patches of yellow coloration. Rhizoctonia could be seen in most of the microlesions. The yellow pigment was apparent around the microlesions where a few cells had been necrosed. The general coloration of the bulbs was due to pigment in patches of cells where no infection was apparent, but the pigmentation was strongest in cells near lesions (Fig. 1,A).

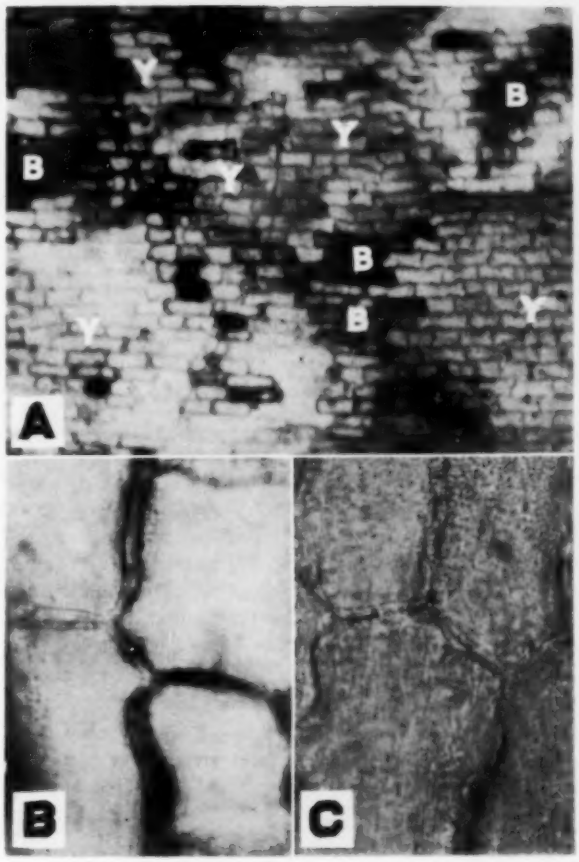


FIG. 1. A) Distribution of lesions and areas of pigmentation on scale of lily bulb. "B" denotes brown cells in and around lesions. "Y" denotes cell areas where lemon-yellow pigment is present (×29). B) Surface view of pigmented cell walls in epidermal cells of lily scale (×387). The apparent thickening of the anticlinal walls as seen from above is an illusion due to the deposition of yellow pigment in the cuticular structures of the periclinal walls where they fold into the anticlinal walls. C) Surface view of nonpigmented cell walls in same scale (×387).

Most of the lemon-yellow pigment was a constituent of the cell walls of the epidermis (Fig. 1,B,C.). This pigment was present only in the cuticle structures of the periclinal walls of the cells. The anticlinal walls of the epidermal cells in yellowed areas often appeared greatly thickened when viewed through the surface of the cells. The thickening is an illusion due to the concentration of pigment at the cornice-like structures where the periclinal walls fold onto the anticlinal walls. The strands of Rhizoctonia present in these areas were often bright lemon-yellow colored like the cell walls, instead of being brown or hyaline.

There was no evidence that the whiteness of the treated bulbs relates directly to chemical action. The

[1] Technical paper No. *1013*, Oregon Agricultural Experiment Station in cooperation with Ornamental Plants Section, Agricultural Research Service, U. S. Department of Agriculture.

[2] Plant Pathologist, Oregon Agricultural Experiment Station, and Agent, U. S. Department of Agriculture.

traces of PCNB on the tiny stem bulblets had no direct contact with the scales of the large bulbs produced by the stem bulblets. Also, stem bulblets planted in heavy soils often produce white bulbs with little indication of Rhizoctonia infection.

The snow-white appearance of bulbs grown from PCNB-treated stem bulblets correlated directly with control of Rhizotonia on the scales, but certain fungi other than Rhizoctonia can produce lesions on the scales of Croft lilies and may likewise lead to a color response. The chemical nature of the pigment and whether it is formed as a protective substance against fungus invasion or as a degeneration product following infection are being investigated.—Department of Plant Pathology, Oregon State College, Corvallis, Oregon.

Streptomycin Foliage Sprays and Control of Bacterial Spot of Peach. ROBERT H. DAINES AND REED GRAY. Bacterial spot of peaches, caused by *Xanthomonas pruni* (E. F. Sm.) Dows., has to date resisted effective control through the use of fungicides. Although foliage sprays of the most effective synthetic compounds will result in about 50 per cent control of fruit infection, the incidence of this disease has at times been sufficient to cause many growers to question the value of such sprays. In 1955, Daines[1] and Goodman and Shepard[2] reported that streptomycin sprays had some promise in the control of bacterial spot.

During the 1956 growing season, an attempt was made to increase the effectiveness of streptomycin by the addition of different materials to sprays containing streptomycin and captan (*N*-trichloromethylthio)-4-cyclohexene-1,2-dicarboxmide). The additions tested were glycerol, a wetting agent (Igepal CA—isooctylphenol + ethylene oxide) and Pyrax (pyrophyllite, a mineral clay with nonexpanding lattice; $Al:SiO_3$ ratio 1:2), which served as a cuticle abrasive. Foliage analysis[3] showed that of the combinations used, the one containing glycerol was the only one that increased streptomycin absorption by the foliage (Table 1). The addition of glycerol also increased the effectiveness of streptomycin against infection by the bacterial spot organism. Old leaves showed considerably more absorbed streptomycin than did young ones.

Disease incidence was much lower where streptomycin was used than where sulfur or captan (2 lb.) was used (Table 1). The difference was significant at the 1 per cent level. Although the streptomycin-glycerol spray, or even the streptomycin as used, are too expensive for commercial adoption, the data presented suggest that considerable control may be achieved by the use of bacteriocides that exert at least limited systemic activity.—Department of Plant Pathology, Rutgers University, New Brunswick, N. J., and Merck, Sharp and Dohme Research Laboratories, Rahway, N. J.

TABLE 1.—*Incidence of bacterial spot on fruit and streptomycin content of leaves from peach trees sprayed with various materials*

Treatment[a]	Percentage of fruits infected[b]		Streptomycin content of leaves (1956)[c]	
	1955	1956	Young	Old
			ppm	ppm
None	31.6			
Wettable sulfur (5 lb.)	24.8	22.6	0.0	1.2
Zinc Omadine[d] (2 lb.)		14.8		
Zinc sulfate (5.5 lb.) + lime (12 lb.)		12.7		
Captan (4 lb.)	9.6	13.5		
Captan (3 lb.)	15.3	19.1		
Captan (2 lb.)	20.0	20.7		
Captan (2 lb.) + streptomycin (150 ppm)	7.5	8.9	4.2	9.7
Captan (2 lb.) + streptomycin (150 ppm) + Igepal CA (10 ml)		9.3	4.3	7.8
Captan (2 lb.) + streptomycin (150 ppm) + Pyrax (2 lb.)		9.5	4.1	8.9
Captan (2 lb.) + streptomycin (150 ppm) + glycerol (1 gal.)		5.7	6.1	15.1
LSD (5% level)	15.3	9.1		
LSD (1% level)	21.1	12.27		

[a] Materials and amounts used per 100 gal. of spray. Treatments consisted of 5 randomized trees. Sprays were applied on a 10-day to 2-week schedule.

[b] Constitution variety used in 1955 and the Sunhigh variety in 1956.

[c] Average of 5 coded samples, each consisting of 30 leaves from 1 tree.

[d] Zinc salt of 2-pyridinethione 1-oxide (50 per cent wettable powder).

Symptoms of Chrysanthemum Flower Distortion, Dodder Transmission of the Virus, and Heat Cure of Infected Plants. PHILIP BRIERLEY AND FLOYD F. SMITH.[1] Chrysanthemum flower-distortion virus[2, 3] was first received in the garden varieties White Wonder and Yellow Spoon from a Pennsylvania nursery in 1952. C. T. Gregory supplied this virus in the florists' varieties

[1] Daines, R. H. 1956. Bacterial spot of peach (Xanthomonas pruni) and its control by the use of captan and streptomycin. Plant Disease Reptr. 40: 335-336.

[2] Goodman, R. N., and P. Shepard. 1956. Indications of Xanthomonas pruni control with antibiotic sprays. Plant Disease Reptr. 40: 93-102.

[3] Gray, R. A. 1956. Increasing the absorption of streptomycin by leaves and flowers with glycerol. Phytopathology 46: 105-111.

[1] Pathologist, Crops Research Division, and Entomologist, Entomology Research Division, respectively, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

[2] Brierley, P. 1955. Symptoms induced in chrysanthemums on inoculation with the viruses of mosaics, aspermy, and flower distortion. Phytopathology **45**: 2-7.

[3] Brierley, P. 1955. Blazing Gold as a test plant for the chrysanthemum flower-distortion virus. Plant Disease Reptr. **39**: 899-901.

FIG. 1. Symptoms of chrysanthemum flower distortion. A–C) Chrysanthemum varieties. A) Infected and normal Indianapolis White. B) Infected and normal Blanche. C) Infected and normal Blazing Gold. D, E) *Vinca rosea*. D) Infected plant. E) Flowers of normal and infected plants.

Indianapolis White, Pink, Yellow, and Bronze from Missouri in 1954. In 1955, J. L. Forsberg supplied similar material in an unspecified florists' variety from Illinois; the identity of the virus in this last sample was not confirmed by transmission, because no suitable scions of the material were available.

The virus is transmissible by grafting but not by the usual mechanical methods or by the fresh-leaf method. On graft inoculation, the chrysanthemum varieties Dauntless, Friendly Rival, Good News, Indianapolis White, and Monument show no specific leaf symptoms initially, but they do produce deformed flowers. Typically, ray florets are short, narrow, and incurved (Fig. 1, A); sometimes a number of rays are suppressed, and the others are short and flat. Affected plants of Dynamo, Golden Mistletoe, Ivory Seagull, Mary MacArthur, and Pandora chrysanthemums are somewhat dwarfed but not rosetted; flowers of these varieties are variously deformed. Of the varieties tested, only Blanche (Fig. 1, B) and Blazing Gold (Fig. 1, C) express symptoms of diagnostic value in vegetative shoots. In these test varieties, leaves at the tips of vegetative shoots are small, yellowish, and misshapen. This symptom is recognizable 2–3 months after grafting—earlier in summer than in winter. Unmistakable rosetting of affected shoots follows; if the plants are permitted to bloom, the typical flower symptoms also appear.

Eventually, the flower-distortion virus is lethal to all chrysanthemum varieties tested. The source varieties White Wonder and Indianapolis White grow weakly; after a few months' growth, roots die and necrosis appears in stem bases. These varieties are maintained by propagation of tip cuttings. W. D. McClellan isolated a species of *Fusarium* from stem bases of declining Indianapolis White, but the fungus isolated was nonpathogenic to this variety or to Blazing Gold. Blazing Gold, when graft-inoculated, develops similar root and stem rot after 5–7 months. Dynamo is more tolerant than Blazing Gold; 1 set of 5 plants of this variety lived 17 months after grafting. Necrosis of roots and of stem bases is believed to be a result of the impaired translocation characteristic of yellows-type viruses.

Seedlings of *Cuscuta campestris* Yuncker were established on Dynamo plants 15 months after the latter were graft-inoculated with flower-distortion virus. The dodder was trained to *Vinca rosea* L. and to healthy Dynamo chrysanthemum. After 2 months' contact,

the Vinca was cut back and the dodder removed. Symptoms of flower distortion appeared in Vinca 6 months after the original dodder connection.

Terminal growth of infected Vinca nearly ceased; lateral shoots developed freely but remained short and leafy (Fig. 1, D). The general effect was a short bushy plant, not a typical broom. Mature leaves turned clear light yellow from tips and margins inward. Branch veins seen from below were often streaked with black. In early stages of infection, a few flowers were produced; these were one-third the diameter of normal blooms, and they were bleached white except at the center—they were never green (Fig. 1, E). In later stages, no flowers appeared. The attempt to transmit the flower-distortion virus from Dynamo to Dynamo by dodder failed.

On May 18, 1956, 4 naturally infected plants of White Wonder and 4 graft-inoculated plants of Dynamo (both varieties with flower-distortion virus) were placed in a greenhouse compartment thermostatically controlled at 35° C night and day. Since no cooling mechanism was provided, the temperature exceeded 35° on some summer days. Tip scions of White Wonder, tested after 2, 3, and 5 months, and scions of Dynamo, tested after 3 months, proved uniformly free of the flower-distortion virus. Testing was by grafting to the Blazing Gold test variety.

The aster yellows virus from Igloo chrysanthemum, collected in Pennsylvania in 1947, also produces rosetting and lethal effects in the variety Blazing Gold, but the onset of these symptoms is later than that of symptoms produced by the flower-distortion virus. Green flowers commonly accompany aster yellows infections in Blazing Gold, but these have never been seen to accompany infections by the flower-distortion virus. *Macrosteles fascifrons* (Stål.) transmitted aster yellows virus from Igloo chrysanthemum to China aster but failed to transmit the flower-distortion virus from White Wonder chrysanthemum to China aster. Symptoms of these 2 viruses in *Vinca rosea* differ widely. Thus, the flower-distortion virus, although clearly a member of the yellows group, is not identical with aster yellows virus.—Agricultural Research Center, Beltsville, Maryland.

Sorghum Grain, a Medium Suitable for the Increase of Inoculum for Studies of Soil-borne and Certain Other Fungi.[1] MARVIN D. WHITEHEAD. Grain sorghum has qualities that make it especially suitable as a medium for increase of inoculum of a wide range of fungi. All the spore-forming genera tested on the medium have made fast growth and have fruited abundantly. The *mycelia sterilia* cover the sorghum kernels with mycelial mats, forming compact inoculum particles. The sorghum medium has been found far superior to cereal grains for obtaining fruiting cultures of the corn-stalk-rotting pathogen, *Diplodia zeae* (Schw.) Lev.

The small round seed form particles of inoculum that will flow freely through drills when it is desirable to apply inoculum with seed in the planting operation. Applying sorghum cultures of known soil-borne pathogens with the seed at planting time has been found to be a satisfactory method for obtaining uniform results in seed-treatment studies with cotton.[2] It is considered an excellent means of applying pathogens to the soil in close proximity to row crops.

Grain sorghum forms an inexpensive, easily prepared medium. White-seeded sorghum is preferred because there are indications that the red-pigmented sorghums may inhibit fungal growth slightly. In the preparation of the medium, seed is soaked in water for approximately 12 hours. If the grain is weathered and covered with soil particles, it may be washed clean easily after soaking. The soaked kernels are placed in 500-ml Erlenmeyer flasks to a depth of about $1\frac{3}{8}$ in. (approximately 160 g of soaked seed). Twenty ml of water is added to the seed, and the flasks are plugged with cotton and autoclaved for 45 minutes at 15 lb. pressure. After cooling, the flasks should be shaken vigorously to separate any grains tending to cling together. The medium should be autoclaved a second time for $1\frac{3}{4}$ hours 24 hours later. Before the reautoclaving, the flasks are checked for moisture content; if the sorghum tends to be drying out on the surface, an additional 10-ml of water is added. Flasks of 500-ml capacity are preferred, but larger sizes have been found satisfactory. When larger flasks are used, a proportionately greater volume of inoculum may be cultured per flask.

When the flasks are cool after the second autoclaving, 4 or more small fragments of agar-plate culture of the desired organisms are placed in each flask and the medium shaken to distribute the inoculum. When the mycelium of the culture begins to cover the medium, the flask should be shaken to break up the mycelium, to prevent matting of the sorghum grain, and to improve the entry of oxygen into the grain culture. This process is repeated as necessary during the first week of incubation of the cultures. The inoculum may be held as pure cultures in the flasks for a considerable period prior to use, or it may be dried and bulked.—Department of Field Crops, University of Missouri, Columbia, Missouri.

[1] Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 1720. Approved by the Director.

[2] Whitehead, M. D., and N. Brown. 1957. In the furrow application of fungicides for the control of cotton seedling disease—damping-off and nub-root. Plant Disease Reptr. (In press)

REPORT OF THE 1957 MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The fourteenth annual meeting of the Potomac Division of the American Phytopathological Society was held February 28, 1957, in the auditorium of the Plant Industry Station, Beltsville, Maryland, and on March 1, 1957, at Fort Detrick, Frederick, Maryland.

Twenty-one scientific papers were presented at Beltsville. On the second day, the Potomac Division membership was welcomed to Fort Detrick by the commanding officer. A symposium "Methods and Measurements in Epiphytology" was presented by the plant pathologists, with Dr. C. H. Kingsolver presiding as chairman. The topics and speakers were 1) General Introduction, C. H. Kingsolver; 2) Spore Deposition, E. L. Sharp, W. E. Miller; 3) Infection and Incubation, R. P. Kahn; 4) Micrometerological Measurements, J. J. Smoot; 5) Air Samplers, J. D. Panzer, R. M. Acker; and 6) Disease Measurements, C. H. Kingsolver.

Following a luncheon, Dr. Per K. Frolich, Deputy Chief Chemical Officer for Scientific Activities, Army Chemical Corps, spoke on the purpose and accomplishments of the Fort Detrick scientific studies.

The officers elected to serve during the next year are as follows: *President*, KERMIT W. KREITLOW; *Vice-President*, L. O. WEAVER; *Secretary-Treasurer*, JOHN G. MOSEMAN; and *Councilor*, JOHN W. HEUBERGER.

L. O. WEAVER, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Hypoxylon punctulatum and its conidial stage. BARNETT, H. L. *Hypoxylon punctulatum* (Berk. & Rav.) Cke. is one of the common fungi found on oak trees killed by oak wilt in West Virginia. The black effused perithecial stromata are formed within the bark, and the outer bark covering is broken off forcibly by means of a ridge of specialized gelatinous tissue that swells and exerts pressure at the edge of the stroma. The thin ectostroma and the gelatinous ridge are thrown off with the bark covering. A conidial fungus belonging to the genus *Basidiobotrys* is often found in stromatic areas before and sometimes after the perithecial stromata appear. The conidial heads are borne on compact fungus pillars, which, by their pressure, form cave-like cracks in the bark. Pure cultures producing the Basidiobotrys conidial stage were obtained from germinating ascospores of *H. punctulatum*, from bits of immature stromata, and from wood beneath the stromata and conidial fructifications. It is concluded that the conidial stage of *H. punctulatum*, unlike most other species of *Hypoxylon*, belongs to the genus *Basidiobotrys*.

Influence of carbon and nitrogen on the growth of Botryosphaeria ribis. BROWN, IRVIN F., JR. Liquid media were used to test the influence of various carbon and nitrogen sources on the growth of *Botryosphaeria ribis* in shake cultures. Sources of carbon were 6 sugars: arabinose, galactose, glucose, fructose, sucrose, and mannitol. Sources of nitrogen were NH_4NO_3, $(NH_4)_2SO_4$, $NaNO_3$, KNO_3, glycine, and asparagine. The amounts of carbon and of nitrogen were uniform in the carbon and nitrogen series, respectively. Wide variation occurred in the utilization, by the fungus, of the carbon and nitrogen in the various sources. In the nitrogen series, maximum mycelial growth (average of oven-dried mat weight in mg of 3 replicates) resulted with the organic sources of glycine (199.3) and asparagine (167.0), followed by KNO_3 (142.3), $(NH_4)_2SO_4$ (75.6), and NH_4NO_3 (66.6); growth was retarded with $NaNO_3$ (29.0). Growth of the check, which contained no added nitrogen, indicated that some nitrogen was carried over in transfer (20.0). In the carbon series, the descending order of mycelial growth for the various sources was fructose (155.5), arabinose (122.5), glucose (114.6), sucrose (113.6), galactose (79.3), and mannitol (57.0). The check, which contained no added carbon, was 0.0. These data indicate that *B. ribis* has definite nutrient requirements for carbon and nitrogen.

Control of phytophthora phaseoli, causal agent of lima bean downy mildew. CROSSAN, D. F., AND P. J. LLOYD. Three resistant bean lines developed by the U.S.D.A. and 4 fungicidal chemicals were tested at 2 different locations for control of *Phytophthora phaseoli* Thaxt. The resistant lines were U.S. 155, 255, and 355 (small-seeded bush type). The fungicides and rates used were maneb (as Manzate, 2-100); nabam plus zinc sulfate (as Dithane D-14 plus zinc sulfate, 2-$\frac{3}{4}$-100); basic copper sulfate (as Tri-Basic, 4-100); and streptomycin (100 ppm) plus 1% glycerol. At 1 location, 3 applications were spaced 7 days apart, beginning when disease was forecast; at the other location, 2 applications were spaced 12 days apart after disease was present. Maneb was very effective in control (less than 1% mildewed pods compared to 38% in the unsprayed); zineb and basic copper sulfate were less effective than maneb but commercially satisfactory. Yield response from maneb was very high. The U.S. resistant lines were essentially free of disease and outyielded Thorogreen. The approach of utilizing a disease forecast for time of fungicidal application permits a minimum of sprays, economical disease control, and high yield.

Changes in spraying practices for banana leaf spot control.—DUNEGAN, JOHN C. Control of the banana leaf spot caused by *Mycosphaerella musicola* Leach with Bordeaux mixture requires an adequate water supply plus the installation of either elaborate stationary spray equipment or the use of mobile spray machines operating over a network of ballasted roads. Such equipment is commercially feasible only in large plantings with level terrain. The oil-mist procedure, introduced by French investigators in the West Indies, does not require water and offers the possibility of protecting small and large plantings, irrespective of terrain, through the use of knapsack sprayers, mist blowers, or helicopters. The small quantity of liquid required (2.5 gal. per acre) and the imperative need to cover the unfolding leaves make the use of helicopters seem especially advantageous. Reports from the Cameroun and from French Guinea indicate adequate coverage if the helicopter flies at the rate of 12–15 miles per hour, for at this slow speed the down draft of the rotor blades completely envelopes the banana plants with mist. Additional tests are being carried on in Jamaica and Ecuador. Widespread adoption of the oil-mist procedures depends on further proof that the oil does not spot the fruit.

Some results of studies on resistance to tobacco black shank. HEGGESTAD, H. E., AND W. B. LAUTZ. Variation in pathogenicity was observed between isolates of the tobacco black shank fungus (*Phytophthora parasitica* var. *nicotianae*). Culture 9-55, maintained since 1952 at Beltsville by reisolation about once each year from diseased plants in resistant lines, was more virulent than cultures recently secured from North Carolina and Florida. Evidence of pathogenic races of the fungus was obtained. The breeding line R-46 (F_2 generation, Dixie Bright 101 × breeding line with resistance to black shank from *N. plumbaginifolia*) was much more resistant than D. B. 101 following inoculation of seedlings at Beltsville with a N. C. isolate and also in a naturally infested field in North Carolina; however, in both greenhouse and field tests at Beltsville, D. B. 101 was more resistant to culture 9-55 than was R-46. Beinhart 1000-1, a selection in a field of cigar variety Quin Diaz collected by E. G. Beinhart in Santa Domingo, has greater resistance to black shank than does the cigar-wrapper variety Rg or than do the present Beltsville breeding lines with resistance from *N. plumbaginifolia*. When certain breeding lines with resistance from *N. plumbaginifolia* were used as pollen parents on Virginia Gold (susceptible), more black-shank-resistant plants were obtained than from the reciprocal hybrids.

Dissemination of sweetpotato cork virus in nature. HILDEBRAND, E. M. The widespread distribution of sweetpotato cork can be attributed to the unrestricted movement of propagation materials. Rapid dissemination within plantings can be ascribed to insect vectors. Dissemination studies in the field at Beltsville, Maryland, during 1955 clarified the life history of the causal virus in relation to pathogenesis. Symptoms on the first "surge of growth" demonstrated that the sprouts from some of the symptomless roots were infected. Although roots were symptomless, the presence of the virus was indicated by the appearance of distinctive chlorotic spots on the surge of foliage growth the first week of July. The appearance of similar spots about 2 months later on hitherto symptomless plants indicated virus spread by insect vectors. Considerable dissemination from row to row within plantings, occurred in all experiments. In 1 plot with 11 rows of 50 plants each, of which rows 3, 6, and 9 were of diseased stock, root slicing after storage demonstrated the following percentages of diseased plants (by rows from north to south): 12, 29, 88, 32, 30, 87, 45, 43, 96, 28, and 13. The greenhouse "flush of growth" technique again revealed that several cork-free roots produced infected plants. Almost 100% dissemination occurred in one 7-row plot containing a lone healthy row.

Freeing sweetpotato varieties from cork virus by propagation with tip cuttings. HILDEBRAND, E. M. Diseased sweetpotato clones were freed from cork virus by the following procedure. 1) One to 5 roots were bedded in sand. 2) Five to 10 sprouts per root were grown singly therefrom in good sweetpotato soil in 4-in. pots in a warm greenhouse for 1 month. (In 3 weeks the "flush of growth" exhibited foliage symptoms if clone was infected.) 3) Next, 2- or 3-node tip cuttings were made from growing plants with symptoms and from symptomless plants; each was bedded separately in sand until rooted. 4) Step 2 was repeated. (The completely symptomless plants were virus-free.) Rooted tip cuttings from symptomless plants all indexed as healthy compared with only 5–50% of those from plants showing symptoms. By this procedure, 7 varieties appear to have been freed from cork virus. These healthy clones were now ready for multiplication—immediately by cuttings or by transplanting into 6-in. pots for root production. The conclusions are supported by several observations: 1) all diseased varieties exhibit foliage symptoms; 2) infected plants rarely show symptoms at tips of terminals; 3) the greenhouse "flush of growth" technique demonstrates virus presence when roots are symptomless; and 4) the rapid indexing method never identifies infected foliage in the absence of symptoms.

Irrigation and disease control: tomato anthracnose and potato seed-piece decay. LLOYD, P. J., AND D. F. CROSSAN. Maneb and zineb were compared on tomatoes under irrigated and nonirrigated conditions. In 1955, irrigated blocks received 5 weekly 1-in. overhead irrigations during a period of limited rainfall; fungicides were applied 1 day before each irrigation. In 1956, 2 irrigations were applied. The results of both tests were as follows: 1) fruit infection by the anthracnose organism was significantly higher under irrigated than under nonirrigated conditions on unsprayed plants; 2) both fungicides gave statistically significant control in both irrigated and nonirrigated blocks; and 3) maneb-treated plants produced significantly fewer infected fruit in the irrigated blocks in 1955 than did those treated with zineb, and the trend was the same in 1956 but the data were barely significant statistically. In the potato seed piece decay (caused by *Erwinia caratovora*) test in 1956, potato seed pieces were cut and treated before planting with captan, 1/100; streptomycin, 100 ppm; streptomycin + captan, 100 ppm + 2/100; and streptomycin + captan, 1% + 2% dust. One irrigation of 1½ in. of water was applied to half of the plots after planting. A significantly lower stand was present in the untreated rows under irrigation than in those not irrigated, and all chemical treatments gave a significantly better stand than did the check (untreated) only under irrigated conditions.

The control of black shank of tobacco with various soil drenches and their effect on the tobacco plant. MORGAN, O. D. Fifty chemicals were screened in the laboratory and greenhouse against *Phytophthora parasitica* var. *nicotianae* over a period of 4 years. Field trials were conducted with 4 of the better fungicides in 1953. Experimental fungicide 5400 (50% α-α-trithiobis (*N*- dimethylthioformamide), Captan 50-W (50% *N*-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide), Dithane D-14 (19% disodium ethylenebis[dithiocarbamate], and CuSOx were used. The field trials were inconclusive. In greenhouse trials, the following proved to be consistently effective as soil drenches: 50% suspensions of the disulfide, iron, manganese and zinc salts of pyridinethione; chloropicrin, experimental fungicide 5400, and Captan 50-W (given in descending order of effectiveness). Stimulation in growth occurred in soil treated with Captan 50-W or with chloropicrin; all others inhibited growth. Assays of leaf sap indicated that the disulfide and manganese salts of pyridinethione moved into the plant. These 2 salts were most phytotoxic when sprayed on the plant tissue.

The effect of various control measures on two parasitic nematodes of strawberry. MORGAN, O. D. Partial control of the root-knot nematode *Meloidogyne hapla* and of species of *Pratylenchus*, a cause of brown root rot, was obtained on the Robinson variety of strawberry by a fall treatment of D-D (1,3-dichloropropene; 1,2-dichloropropane) at 60 gal. per acre; by a heat treatment of half the plants in the test area; and by applications of a dry formulation containing 20% Nemagon (1,2-dibromo-3-chloropropane) at rates of 68 and 136 lb. per acre side dressed on 5 replicated rows of both heated and nonheated blocks. The mother plants were treated, and about 250 daughter plants were checked for nematode damage. There was significantly less root knot in the Nemagon treatments than in the checks without Nemagon. The percentages of plants with root-knot in the heated block in the high- and low-Nemagon plots and in the check plots without Nemagon were 1.64, 0.81, and 42.4%, respectively. The percentages of rootknot in the nonheated block were 2.8, 2, and 66.7, respectively. There was significantly less brown root rot in both heated and nonheated blocks treated at the high rate of Nemagon than in the checks. There were fewer Pratylenchus in the Nemagon treatments than in the checks.

Host-parasite interactions between culture 12A1 of the powdery mildew fungus and the Ml_k and Ml_a genes in barley. MOSEMAN, JOHN G. The interaction of the genes conditioning resistance to *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal in the barley varieties Kwan and Goldfoil and the genes for pathogenicity in culture 12A1 of Race 12 on these varieties was determined. The relationship of genes conditioning resistance to culture CR3.1 of Race 3 and culture 21.1-3 of Race 8 was studied in varieties Kwan and Goldfoil. Results indicate that the varieties Kwan and Goldfoil each has 1 gene conditioning resistance to culture CR3.1, which is a single-spore culture from California Race 3. The same genes also were found to condition resistance to culture 21.1-3. The genes conditioning resistance to California Race 3 in these 2 varieties are inherited independently, and they have been designated Ml_k and Ml_a. Inheritance of factors for pathogenicity on the varieties Kwan and Goldfoil was determined by studying the progeny of a cross of cultures 12A1 and 21.1-3. Culture 12A1 has 1 gene for pathogenicity on each of the 2 varieties. These 2 genes were inherited independently. There were 2 independently inherited genes, Ml_k and Ml_a, in the varieties Kwan and Goldfoil, respectively, that condition resistance to powdery mildew, and 2 independently inherited genes for pathogenicity in Culture 12A1, one for each variety.

Mineral salts as related to plant virus local lesions. PANZER, JAMES D. Since the present trend toward systemics in plant virus work has gained impetus, much work is needed on the preinoculation environment of the assay plants. A simple Mason-jar hydroponic apparatus was used in testing the effects of mineral salts on lesions on Pinto beans inoculated with tobacco mosaic virus (TMV). No lesions developed when Pinto bean seeds were germinated in solutions of mineral salts and the seedlings subsequently transferred to distilled water prior to inoculation with TMV. Lesions developed on inoculated plants exposed continuously to mineral salt solutions; the greatest number occurred on plants grown in a complete Meyer's solution and progressively fewer on plants grown in solutions devoid of K, P, and N. Lesion number was reduced indirectly through the precipitation of mineral salts when a slightly acid tap water was used. No lesions developed when plants were grown only in single-salt solutions of N, P, K, or Fe or in a micrometabolic-element solution. In a cation series, greatest lesion number occurred in the area of high Mg with low K and Ca and in the area of high Ca with low Mg and K. In an anion series, greatest lesion number occurred in the area of high PO_4 with low NO_3 and SO_4.

A simple 24-hour spore collector. PANZER, JAMES D., E. P. VANARSDEL, AND EDGAR C. TULLIS. A simple, low cost, 24-hour spore collector that is adequate for field or laboratory use was developed. The collector consists of an airtight wooden box with a 6-volt automobile fan mounted in a hole in the top. The fan forces air from the box so that the resulting negative pressure causes spore-laden air to be drawn into the box through a small piece of copper tubing. Standard microscope slides are mounted vertically on the metal table of a Taylor dew meter in such a way that the air stream impinges on a small area of 1 slide each hour. Glycerol-coated slides have proved successful, but any adhesive may be used, depending on the pathogen. The slide holder is powered by a 24-hour clock and the fan by a 6-volt automobile wet-cell battery, which will easily last for 24 hours. Twenty-four-hour collection curves for total spore count of the air and for spore counts of *Leptosphaeria* sp., *Piricularia oryzae*, and *Alternaria* sp. were obtained by use of this device.

Histology of wheat plants infected with the powdery mildew fungus and treated with anisomycin. POWERS, H. R. JR. Anisomycin, an antibiotic isolated from 2 species of *Streptomyces*, eradicated *Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal on wheat when applied in an aqueous solution at 300 ppm, provided coverage was thorough. Eradication was accomplished by spraying as late as 7 days after inoculation, by which time the mildew was well developed. The anisomycin primarily affected the external mycelium and was very rapid in its action. Microscopic examination of fixed and stained leaf sections, taken 2 hours after spraying, revealed that the surface mycelium was plasmolyzed. The affected mycelia were also heavily stained with safranin, which indicated necrosis. In contrast, the haustoria parasitizing the epidermal cells directly under the injured mycelia were similar in appearance to those examined on untreated leaves. This indicated that either the haustoria were more resistant to the antibiotic than was the external mycelium or that the anisomycin was not systemic. The latter would seem to be the more likely conclusion.

Genetics of host-parasite relationship in powdery mildew of wheat. POWERS, H. R., JR., AND W. J. SANDO. The relationship between 2 independent, dominant genes conveying mildew resistance in the host and 2 genes for virulence in the pathogen was investigated. The wheat variety Normandie, which has been shown to carry the genes Ml_1 and Ml_a for mildew resistance, was crossed with Little Club wheat, which has the recessive alleles. Cultures of *Erysiphe graminis* f. sp. *tritici* used were 116-3, virulent against Ml_1 but not against Ml_a, and 116-2, with the reverse combination. Neither culture could attack Normandie, but both could attack Little Club. These 2 cultures were used to inoculate the F_2 and F_3 plants from the cross Normandie $\times$ Little Club. The 4 possible homozygous genotypes of the host were identified by these tests. These genotypes were then used to identify the 4 possible haploid genotypes of the pathogen resulting from a cross between cultures 116-2 and 116-3. These were the 2 parental types, a third type more virulent than either parent (since it could attack Normandie), and a fourth type capable of attacking only Little Club. The more pathogenic new type therefore contained both genes for virulence whereas the fourth type had neither. These results indicate that for each of the genes Ml_1 and Ml_a in the host there is a complementary gene for virulence in the pathogen.

Temperature as it affects growth of apple fruit-rotting organisms and their response to fungicides in agar-plate tests. SAYED, M. Q., AND J. W. HEUBERGER. The growth response of *Botryosphaeria ribis*, *Physalospora obtusa*, and *Monilinia fructicola* at a range of temperatures (12°, 20°, 28°, 34°C) was determined in agar-plate tests. Optimum for each organism was about 28°C. Best growth range was 20°–28°C for *P. obtusa* and *M. fructicola*, whereas for *B. ribis* it was 20°–34°C. *B. ribis* grew more vigorously than did *P. obtusa*, which grew more vigorously than did *M. fructicola*. The descending order of fungicidal activity of captan (as Captan 50-W), zineb (as Dithane Z-78), and captan + zineb (half-strength of each) when incorporated in the agar against each organism was as follows: *B. ribis*—captan + zineb, zineb, captan; *P. obtusa*—captan, captan + zineb, zineb; 100 *M. fructicola*—captan, captan + zineb, zineb. When ED100 points for each fungicide at each temperature were plotted, it was found that the fungicides were least effective at the optimal temperatures for growth of each organism and most effective at either the minimal or maximal temperatures.

Viability of Rhizoctonia and Aphanomyces cultures kept under mineral oil and sealed with Parafilm. SCHNEIDER, C. L. Transfers were recently made of test tube cultures of *Rhizoctonia* species., including many isolated from potato and sugar beet, and of *Aphanomyces cochlioides;* the cultures had been collected by the late Dr. J. E. Kotila during the period from 1932 to 1950. All of the Rhizoctonia cultures and most of those of Aphanomyces were on slants of potato-dextrose agar, usually amended with vitamins B_1 and C. Some cultures of *A. cochlioides* were on maize-meal agar. Most of the cultures had been covered with sterile mineral oil, and each tube was sealed by covering the cotton plug with Parafilm. The cultures were stored on shelves in

a laboratory at the Plant Industry Station, Beltsville, Maryland, where the temperatures ranged from about 65° to 95°F. Of 76 cultures of Rhizoctonia, 6 to 7½ years old, covered with mineral oil and sealed with Parafilm, 70 were viable; of 40 cultures 5½ to 9 years old, sealed with Parafilm alone, 31 were viable. With cultures of *A. cochlioides* 7 to 7½ years old, covered with mineral oil and sealed with Parafilm, 11 of 15 were viable.

The influence of nitrogen and potassium on the relationship of Meloidogyne incognita acrita and soybeans. SHANDS, W. A., JR., AND H. W. CRITTENDEN. The soybean varieties Anderson (resistant) and Adams (susceptible) received 3 levels of nitrogen. The soybean varieties Anderson, Wabash (moderately susceptible), and Adams received 3 levels of potassium. A uniform amount of inoculum of *Meloidogyne incognita acrita*, a root-knot nematode, was used in all tests. A uniform amount of a legume bacterial inoculum was incorporated into certain of the nitrogen tests and into all of the potassium tests. Duration of greenhouse pot tests was 42 days. Percentages of galls on Adams and Wabash were obtained. Galls were not present on Anderson; therefore, roots were stained with Flemming strong fixative to show the presence of larvae. Nitrogen and potassium influenced the relationship of *M. incognita acrita* and soybeans; there was a general increase in penetration of Anderson and in the amount of galls on Adams and Wabash with an increase of both nitrogen and potassium. The data from the nitrogen tests indicate that in the presence of legume bacteria the amount of galls on Adams and penetration of Anderson by larvae of this nematode is increased.

Virus diseases of iris. TRAVIS, R. V. Three virus diseases of iris have been described, namely 1) bulbous iris mosaic, 2) bearded iris mosaic, and 3) beardless iris mosaic. Two additional ones, 4) mosaic of *Iris fulva* and 5) iris ringspot, are reported here. All 5 causal viruses are transmissible by mechanical inoculation; those causing 2, 3, and 4 infect *Belamcanda chinensis*, but those causing 1 and 5 do not. *Myzus persicae* transmits the viruses causing 1, 2, 3, and 4; *Macrosiphum solanifolii* transmits those of 1, 3, and 4; and *Aphis fabae* transmits those of 3 and 4. All but iris ringspot virus are relatively unstable and are transmitted in the nonpersistent manner. Only iris ringspot virus infects Bountiful bean, Samsun tobacco, *Chenopodium amaranticolor*, *Gomphrena globosa*, guar, *Physalis angulata*, and *Amaranthus retroflexus;* it is infectious after heating for 10 minutes at 62° C, after dilution to 10^{-3}, and after aging at 20° for 10 days. There was no evidence of cross protection between the iris ringspot virus and carnation ringspot, tomato ringspot, cucumber mosaic, tobacco etch, alfalfa mosaic, or potato X viruses. Bearded iris mosaic virus was isolated from 4 different species of iris, and those causing beardless iris mosaic, mosaic of *Iris fulva*, and iris ringspot each were isolated from 1 species of iris.

New snap and Pinto beans resistant to several diseases. ZAUMEYER, W. J., AND H. REX THOMAS. A new mosaic-resistant green-podded bush snap bean, temporarily designated as 1831-9, and Columbia Pinto, a new multiple disease-resistant dry bean, are being released to seedsmen and growers, respectively. The parents of 1831-9 are Topcrop, a mosaic-resistant variety, and Tenderpod. It is immune from 3 virus diseases—common bean mosaic, that caused by the New York 15 strain of common bean mosaic virus, and pod mottle. Its performance records show that it is widely adapted and a dependable bearer. Columbia Pinto not only incorporates the disease resistance of the present-day varieties of Pinto bean—resistance to common bean mosaic and to curly top—but also it is resistant to the New York 15 strain of bean mosaic virus, and to several races of the bean rust pathogen. The new variety was developed jointly by the U. S. Department of Agriculture and the Washington and Idaho Agricultural Experiment Stations. It is the result of a cross between Pinto 5, a rust-resistant type, and Great Northern 123, with later outcrossings to Pinto 78. Its seeds are attractive and larger than those of the commercially grown Pinto varieties. This new bean is adapted to the irrigated districts of the Mountain States.

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

JACOBS, M. D., M. J. GERSTEIN, AND W. G. WALTER. 1957. **Dictionary of microbiology.** D. Van Nostrand Co., Princeton, N. J., New York, Toronto, London. 276 p. Price $6.75.

This dictionary defines more than 5000 terms commonly used in microbiology and the related fields of bacteriology, mycology, virology, cytology, immunology, serology, and microscopy. The entries include such substances as antibiotics, sera, chemical compounds, and enzymes. Important culture media, culture methods, stains, staining procedures, diagnostic tests, and types of apparatus are covered in detail. Descriptions are given for the most important bacteria, fungi, viruses, yeasts, rickettsiae, and protozoa. When possible, the authors have followed the system of nomenclature and classification according to Bergey's "*Manual of Determinative Bacteriology*."

SMITH, K. M. 1957. **A textbook of plant virus diseases.** Ed. 2. Little, Brown and Company, Boston. 652 p. 94 illustrations. Price $12.

The publication of this thoroughly rewritten text, 20 years after the date of appearance of the first edition, will serve as another milestone in the development of virology in English-speaking countries and indeed widely throughout the world. Practically all the viruses known to affect plants are included. Treatment is encyclopedic, individual viruses being treated in the alphabetic order of their best recognized common names. An index of synonyms appears at the end of the book, but no general index to the naturally and experimentally infected host plants and insect vectors mentioned in the text is included.